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The health effect of primary concern with regard to exposures of humans to toluene is dysfunction of the central nervous system (CNS). Occupational exposures in the range of 200 to 1,500 ppm have elicited dose-related CNS alterations. Although myelotoxicity was previously attributed to toluene, recent evidence indicated that toluene is not toxic to the blood or bone marrow; myelotoxic effects are considered to have been the result of concurrent exposure to benzene.

Available evidence is inadequate for assessing the carcinogenic potential of toluene. Although a 24-month inhalation exposure of rats to 300 ppm did not produce any positive carcinogenic effects, various design deficiencies precluded the usefulness of this study in assessing carcinogenic potential.

Toluene has been shown to be non-mutagenic in a battery of microbial, mammalian cell, and whole organism test systems. Animal exposure studies suggest that toluene has low teratogenic potential. However, embryotoxicity has been shown to be an endpoint of concern. The reproductive effects of toluene is a category recommended for additional research.

Based on available exposure estimates, the only group at possible high risk are workers exposed at or near the Threshold Limit Value (100 ppm).

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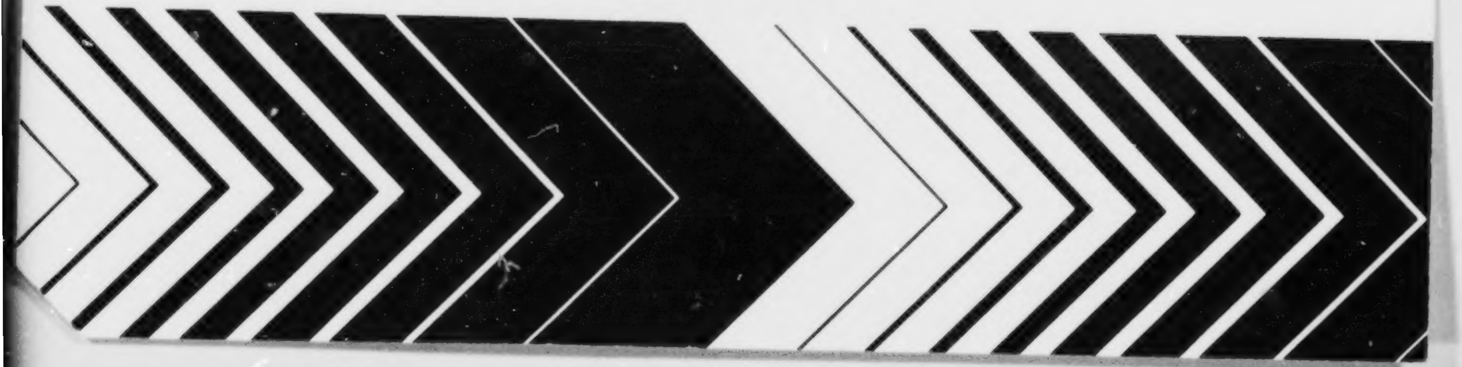

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FINAL
REPORT

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
Research Triangle Park, NC 27711

PREFACE

This document has been prepared by the Environmental Criteria and Assessment Office of the U.S. Environmental Protection Agency (EPA). The document was originally developed to support U.S. EPA decision-making regarding possible regulation of toluene as a hazardous air pollutant. The scope of the document has since been expanded to address multimedia aspects and thus enables the document to serve as a "source document" for other U.S. EPA programs requiring comprehensive information concerning the health effects of toluene.

The Health Assessment Document for Toluene was reviewed and critiqued by the Environmental Health Committee of the U.S. EPA Science Advisory Board in August 1982. This committee provides advice on scientific matters to the Administrator of the U.S. Environmental Protection Agency.

In the development of the assessment document, the scientific literature has been critically evaluated and the conclusions presented in such a manner that the toxicity of toluene and related characteristics are qualitatively identified. Observed effect levels and other measures of dose-response relationships are discussed, where appropriate, in order that the nature of the adverse health responses are placed in perspective with observed environmental levels.

ABSTRACT

Toluene is the most prevalent hydrocarbon in the atmosphere. Levels generally range from 0.14-57 ppb. Levels in water generally are below 10 ppb. Gasoline usage and automobile exhaust represent the largest atmospheric source. Over 3 million metric tons of toluene are produced annually in the United States.

Available evidence associated with effects upon humans and experimental animals indicates that the health effect of primary concern is dysfunction of the central nervous system (CNS). However, observed effects are associated with exposure levels greatly in excess of those levels in the environment. Dysfunction of the CNS may occur during short-term (≤8 hours) exposure to 100-300 ppm.

Toluene has not demonstrated any overt signs of kidney or liver damage upon animal experimentation. It was non-carcinogenic in rats exposed to 300 ppm for 24 months. However, the full extent of toluene's carcinogenic potential is currently being evaluated, at higher exposure levels, in a lifetime bioassay of rodents in the National Toxicology Program. Toluene is classified as provisionally non-mutagenic, and its teratogenic potential has not been fully explored.

The results of the available evidence indicate that exposure to environmental levels of toluene is unlikely to constitute a significant hazard to the general population.

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AUTHORS, CONTRIBUTORS, AND REVIEWERS

This health assessment document for toluene was prepared by the Syracuse Research Corporation under contract with U.S. EPA Environmental Criteria and Assessment Office (Mark Greenberg, Project Manager).

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Chapter 1: Executive Summary

Chapter 2: Introduction

Chapter 3: Physical and Chemical Properties
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Chapter 4: Production, Use, and Releases to the Environment
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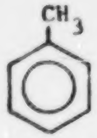
1. EXECUTIVE SUMMARY

1.1. ENVIRONMENTAL SOURCES, FATE, AND LEVELS

Toluene, a homolog of benzene that contains a single methyl group, is a clear, colorless liquid at room temperature. The molecular formula of toluene is C_7H_8 and the molecular weight is 92.13. The structural formula is given below.



Other physical properties of toluene include a melting point of -95°C, a boiling point of 110.6°C, a flash point of 4.44°C, a vapor pressure of 28.7 torr at 25°C, and a density of 0.8669 g/mℓ at 20°C. Toluene is slightly soluble in both fresh and salt water (535 mg/ℓ and 379 mg/ℓ, respectively) at a temperature of 25°C. The physical properties of toluene indicate that toluene in the environment is likely to be present in the air, and that toluene originally present in water may be transferred to the atmosphere. Toluene can undergo photochemical reactions, particularly under atmospheric smog conditions. In aqueous media under the conditions of water chlorination, toluene may be chlorinated followed by subsequent hydrolysis to benzaldehyde. This reaction may account for the benzaldehyde detected in some finished drinking waters.

The general population may be exposed to toluene through inhalation of air, ingestion of food or water, or dermal exposure. The four largest sources of emission of toluene to the atmosphere are, in descending order of importance, automobile use, industrial use of toluene as a solvent, coke ovens, and toluene-producing industries. In addition to air, toluene has been detected in drinking water and in the flesh of edible fish. Dermal exposure to toluene occurs primarily in the workplace. The estimated quantities of toluene taken in by the general public from each source are between a trace and 94 mg/week by inhalation (depending on whether an individual resides in an urban or rural area or near an industry that uses toluene) and 0.0 to 0.75 mg/week from food and water. Occupational exposure (up to 18,000 mg/week) or cigarette smoking (14 mg/week from 140 cigarettes) will increase an individual's exposure to toluene. Although there are technical problems with estimating inhalation exposure to toluene, there is reasonable agreement between the values obtained by dispersion modelingand those obtained from calculations using monitoring data. It should be noted that the exposure scenario discussed above does not account for inhalation and dermal exposure to toluene from gasoline during vehicular filling operations, or from solvents or other toluene-containing consumer products. No quantitative estimate of either the number of people exposed or the extent of exposure can be provided for these sources, although consumer usage could contribute significantly to total exposure.

The total amount of toluene produced in the United States in 1978 was 3595 million kg. The majority (96.5%) is produced by catalytic reformation from selected petroleum fractions, and the remainder is produced from pyrolytic cracking, and as a recovered by-product of styrene production and coke oven emission. This value of 3595 million kg is for isolated toluene and accounts for only 11% of the total toluene produced; the remaining 89% of the toluene produced is not isolated as pure toluene but is a benzene-toluene-xylene mixture used in gasoline. Toluene is also used as feed stock for the production of benzene and other chemicals, as a gasoline additive, and as a solvent.

Activities associated with automobiles (marketing and evaporation of gasoline and automobile exhaust) are the largest single atmospheric source of toluene release (677 million kg/year), with industries using toluene as a solvent (the paint and coating, adhesive, ink, and pharmaceutical industries) being the second largest source of toluene in the atmosphere (375 million kg/year). These two sources account for 75% of the toluene emitted to the atmosphere. The amount of toluene released to other media in the environment is small and is equal to approximately 0.15% of the total amount released to the atmosphere.

The preferred method for the monitoring of toluene in ambient air consists of sorbent collection, thermal elution, and GC-FID determination. For a 25 ℓ sample, the detection limit is <0.1 ppb (0.38 $\mu g/m^3$). Purge and trap with GC-photoionization detection is the most widely used method for the analysis of toluene in aqueous samples. With a 5 mℓ sample, the method has a detection limit of 0.1 ppb (0.1 $\mu g/\ell$).

Toluene is the most prevalent aromatic hydrocarbon in the atmosphere, with average measured levels ranging from 0.14 to 59 ppb (≈0.53 to 200 $\mu g/m^3$). Toluene has also been detected in surface waters and in treated wastewater effluents at levels generally below 10 ppb (10 $\mu g/\ell$). A concentration of toluene as high as 19 ppb (72 $\mu g/\ell$) has been detected in a drinking water supply. In a study of toluene levels in the tissue of edible aquatic organisms, 95% of the samples contained less than 1 ppm (1 mg/kg) of toluene. The atmosphere is the major environmental receiver for toluene. It has been estimated that approximately 124 million people in the U.S. are exposed to atmospheric toluene at a concentration level greater than 0.27 ppb (1.0 μg/m^3).

Toluene released to the aquatic or soil environment is at least partly removed by biodegradation. There is little information on the rate and extent of biodegradation in soil; however, in one study, a half-life of between 20 and 60 min was observed in soil containing toluene-degrading bacteria, and in a second study 20 to 60% of toluene was removed following percolation through 140 cm of sand. Because of the limited number of studies available, the extent of toluene degradation in soil cannot be determined, although studies with pure cultures indicate that a variety of bacteria and fungi can use toluene, and some pure cultures have been isolated that can use toluene as a sole source of carbon. Toluene is also readily biodegraded in aqueous media, both in surface water and during wastewater treatment; however, disappearance of toluene from aqueous media is mainly through evaporation and transport to the atmosphere. The conversion of toluene to compounds that can be used as sources of carbon and energy suggests that toluene will be degraded rapidly by microbial species proliferating at the expense of the compound, and will not accumulate significantly in the environment.

1.2. EFFECTS ON HUMANS

Toxicity studies of humans have primarily involved evaluation of individuals exposed to toluene via inhalation in experimental or occupational settings or during episodes of intentional abuse. The results of these studies indicate that although people exposed occupationally may be at risk, exposure to ambient levels of toluene is not likely to constitute a significant hazard to the general population.

The health effect of primary concern is dysfunction of the central nervous system (CNS). Acute experimental and occupational exposures to toluene in the range of 200 to 1500 ppm (≈750 to 5600 mg/m^3) have elicited dose-related CNS alterations such as fatigue, confusion, and incoordination, as well as impairments in reaction time and perceptual speed. Following initial CNS excitatory effects (e.g., exhilaration, lightheadedness), progressive development of narcosis has characterized acute exposures to excessive concentrations of toluene (i.e., levels approaching the air saturation concentration of approximately 30,000 ppm (≈113,000 mg/m^3)). Repeated occupational exposures to toluene over a period of years at levels of 200 to 400 ppm (≈750 to 1500 mg/m^3) have resulted in some evidence of neurologic effects, and chronic exposure to mixtures of solvent vapors containing predominantly toluene at levels of 30 to 100 ppm (≈100 to 400 mg/m^3) have resulted in impaired performance on tests for intellectual and psychomotor ability and muscular function. Prolonged abuse of toluene or solvent mixtures containing toluene have, on occasion, led to residual or permanent CNS effects.

Early reports of occupational exposures ascribed myelotoxic effects to toluene, but the majority of recent evidence indicates that toluene is not toxic toward the blood or bone marrow. The myelotoxic effects previously attributed to toluene currently are considered to have been the result of concurrent exposure to benzene, which was typically present as a contaminant. Acute exposures to toluene have not resulted in any definite effects on heart rate or blood pressure.

Liver enlargement was reported in an early study of painters exposed to 100-1100 ppm (≈400 to 4100 mg/m^3) toluene for 2 weeks to more than 5 years, but this effect was not associated with clinical evidence of liver disease or corroborated in subsequent studies. Chronic occupational exposure to toluene or intensive exposure via glue or thinner sniffing generally has not been associated with abnormal liver function. Evidence of renal dysfunction has been observed in workers who were accidentally overexposed to toluene and in toluene abusers, but studies of workers exposed to 100 to 1100 ppm (≈400 to 4100 mg/m^3) toluene for 2 weeks to 5 years and 60 to 100 ppm (≈200 to 400 mg/m^3) toluene for over 3 years did not report abnormal urinalysis findings. Several reports have appeared recently that associate deliberate inhalation of toluene with metabolic acidosis.

Subjective complaints of dysmenorrhea have been reported by women exposed for over 3 years to 60 to 100 ppm (≈200 to 400 mg/m^3) toluene and concommitantly to 20 to 50 ppm gasoline in a "few" working places. Disturbances of menstruation have also been reported in female workers exposed concurrently to toluene, benzene, xylene, and other unspecified solvents. The limited available data do not, however, specifically associate occupational exposure to toluene with menstrual effects. Information on the possible reproductive effects of toluene in males is not available.

Single short-term exposures to moderate levels of toluene have, on occasion, been reported to cause transitory eye and respiratory tract irritation, but irritative effects have generally not been observed in workers exposed repetitively to toluene. Dermal contact with toluene may cause skin damage due to its degreasing action.

1.3. ANIMAL STUDIES

The most pronounced effect of toluene in animals is on the CNS. Acute exposure via inhalation to high levels of toluene has been associated with depression of activity. Levels below 1000 ppm (≈3800 mg/m^3) have little or no effect on gross manifestations of behavior, although more sensitive methods of assay (i.e., detection of changes in cognition and brain neuromodulator levels) have indicated effects at lower levels.

Early studies with animals suggested that toluene induced myelotoxicity, but most studies that used toluene that contained negligible amounts of benzene have not produced injury on blood-forming organs.

Inhalation of concentrations of up to 1085 ppm (≈4100 mg/m^3) toluene for 6 weeks or 300 ppm (≈1100 mg/m^3) for 24 months, or ingestion of 590 mg toluene/kg body weight/day for 6 months produced no evidence of liver damage; however, several studies noted an increased liver weight or slight histological changes suggestive of possible liver damage at higher levels of vapor exposure (≈2000 ppm (≈7500 mg/m^3) in rats), or in animals treated by the intraperitoneal route (≈0.4 g/kg).

Renal injury was noted in rats, dogs, and guinea pigs after subchronic inhalation of toluene vapors at levels in excess of 600 ppm (≈2300 mg/m^3) in three studies, but other subchronic exposures in which rats, dogs, guinea pigs, and monkeys inhaled (concentrations up to 1085 ppm (≈4100 mg/m^3)) or ingested (590 mg toluene/kg body weight/day) toluene did not produce renal damage.

Although no effect was observed on the lungs of rats, guinea pigs, dogs, or monkeys after intermittent exposure to 1085 ppm (≈4100 mg/m^3) toluene vapor for 6 weeks, in rats after inhalation of up to 300 ppm (≈1100 mg/m^3) toluene for 24 months, or in rats after ingestion of 590 mg toluene/kg body weight/day for 6 months, other studies have noted irritation effects in the respiratory tract in dogs, guinea pigs, and rats. Sensitization of the heart in mice, rats, and dogs has been associated with inhalation of toluene.

The acute oral toxicity (LD_{50}) of toluene in rats is in the range of 6.0 to 7.5 g/kg, which indicates only slight toxicity in this species. Inhalation LC_{50} values have been reported in the range of 500 to 700 ppm (≈1900 to 2600 mg/m^3) for mice and 4000 ppm (≈15,000 mg/m^3) for rats. Acute dermal toxicity appears to be quite low (rabbit LD_{50} of 12.2 g/kg), but slight to moderate irritation has been noted in rabbit and guinea pig skin and in rabbit corneas after application to the skin and eye, respectively.

1.4. ABSORPTION, DISTRIBUTION, METABOLISM, ELIMINATION, AND RELATED PHARMACOKINETICS

Toluene is readily absorbed from the respiratory tract. Studies with humans indicate that the total amount of toluene absorbed is proportional to the concentration of toluene in inspired air, the length of exposure, and pulmonary ventilation, which in turn depends upon the level of physical activity. Approximately 50% of the amount inspired is retained in the body. Absorption of toluene from the gastrointestinal tract is probably fairly complete, based on excretion data from experimental animals. Toluene is absorbed less readily through the skin than through the respiratory or gastrointestinal tracts.

Animals given toluene orally or by inhalation had high concentrations of toluene in their adipose tissue and bone marrow, and moderately high concentrations of toluene and its metabolites in liver and kidney. These results are reasonable based on tissue-blood partition coefficients and known routes of metabolism and excretion.

The initial step in the metabolism of toluene is side-chain hydroxylation by the hepatic mixed-function oxidase system, followed by oxidation to benzoic acid. Benzoic acid is then conjugated with glycine to form hippuric acid and excreted in the urine. In both humans and animals, 60 to 75% of the absorbed toluene can be accounted for as hippuric acid in the urine, regardless of the dose or whether the chemical was administered orally or by inhalation. Much of the remaining toluene is exhaled unchanged. The excretion of toluene and its metabolites is rapid; the major portion occurs within 12 hours of oral administration or the end of inhalation exposure.

1.5. CARCINOGENICITY, MUTAGENICITY, AND TERATOGENICITY

Inhalation exposure to toluene at concentrations of up to 300 ppm ($\approx$1100 mg/m^3) for 24 months did not produce an increased incidence of neoplastic, proliferative, inflammatory, or degenerative lesions in various organs of rats relative to unexposed controls. It should be noted, however, that the 300 ppm ($\approx$1100 mg/m^3) exposure did not represent a maximum tolerated dose. A bioassay of commercial toluene in rats and mice exposed via inhalation is currently being conducted by the National Toxicology Program Carcinogenesis Testing Program. Other studies indicate that toluene is not carcinogenic when applied topically to the shaved skin of laboratory animals and that it does not promote the development of skin tumors following initiation with 7,12-dimethylbenz[a]anthracene.

Toluene has been shown to be non-mutagenic in a battery of microbial, mammalian cell, and whole organism test systems, but assays for sister-chromatid exchange (SCE) and cytogenetic effects have provided conflicting results. Increased frequencies of SCEs and/or chromosome aberrations were found in some, but not all, studies of lymphocytes from workers who were chronically exposed to toluene, and SCEs and/or aberrations were not induced in Chinese hamster ovary cells or human lymphocytes exposed to toluene in culture. Russian studies have reported chromosome aberrations in the bone marrow cells of rats exposed subcutaneously and via inhalation to toluene, but these findings have not been corroborated in other studies of rats following intraperitoneal injection of toluene.

Toluene has been reported to induce cleft palates and embryotoxic effects in mice following oral exposure, but it was not teratogenic in mice or rats following inhalation exposure. Embryotoxic effects (increased incidence of skeletal anomalies and signs of retarded skeletal development, low fetal weights) and increased maternal toxicity were noted, however, in some of the rats and mice exposed via inhalation.

1.6. EFFECTS ON ECOSYSTEMS

The ecological effects of toluene have been investigated using aquatic and terrestrial microorganisms, aquatic invertebrates, fish, and higher plants. Toluene can both stimulate and inhibit growth of bacteria and algae, depending on the species and the concentration of toluene. The growth of most species of bacteria, algae, and other microorganisms is not inhibited until the toluene concentration exceeds 10 to 100 mg/ℓ. Toluene is acutely toxic to aquatic invertebrates and fish at concentrations ranging between 3 and 1180 mg/ℓ. The lowest concentration shown to cause sublethal effects in aquatic animals was 2.5 mg/ℓ. Chronic toxicity data are available for two species of fish; marine sheepshead minnows were affected at toluene concentrations of 7.7 mg/ℓ but not at 3.2 mg/ℓ, and fathead minnows were affected at 6 mg/ℓ but not at 4 mg/ℓ. Chronic effects occurred at concentrations that were about 2 to 18 times lower than the acute LC_{50} for these species, indicating that chronic effects may occur at lower levels in more sensitive species. Toluene concentrations between 0.1 and 1.0 ppm have been reported occasionally in surface waters (0.1 to 1.0 mg/ℓ) and sediments (0.1 to 1.0 mg/kg). These concentrations are sufficiently close to the toxic concentrations for sensitive species to indicate that acute or chronic toxic effects may occur in some polluted habitats, especially after accidental spills of toluene. Toluene has only a low bioconcentration potential and is metabolized and rapidly depurated from fish, which indicates that toluene is unlikely to biomagnify through aquatic food webs. Toluene, however, has been shown to impart an unpleasant taste to fish that inhabit contaminated water. The impact of toluene spills or chronic low-level pollution on ecosystems is unknown. Adverse effects may occur but probably are limited by rapid rates of loss of toluene through evaporation and biodegradation.

1.7. HEALTH EFFECTS SUMMARY

Considerable information is available on the effects of toluene on humans and experimental animals after inhalation exposures. The data on oral exposure are much less satisfactory, although one acceptable subchronic oral study using rats is available. No information on dermal exposures suitable for use in human risk assessment was encountered.

Based on a few studies involving controlled exposures of humans to toluene vapors as well as some reports of occupational incidents and voluntary abuse ("glue sniffing"), the dose-response relationships for the acute effects in humans of single short-term exposures to toluene can be estimated as:

10,000 to 30,000 ppm (≈38,000 to 113,000 mg/m^3) : Onset of narcosis within a few minutes. Longer exposures may be lethal.

>4,000 ppm (≈15,000 mg/m^3) : Would probably cause rapid impairment of reaction time and coordination. Exposures of one hour or longer might lead to narcosis and possibly death.

1,500 ppm (≈5600 mg/m^3) : Probably not lethal for exposure periods of up to eight hours.

300 to 800 ppm (≈1100 to 3000 mg/m^3) : Gross signs of incoordination may be expected during exposure periods up to eight hours.

400 ppm (≈1500 mg/m^3) : Lacrimation and irritation to the eyes and throat.

100 to 300 ppm (≈400 to 1100 mg/m^3) : Detectable signs of incoordination may be expected during exposure periods up to eight hours.

200 ppm ($\approx$750 mg/m^3) : Mild throat and eye irritation.

50 to 100 ppm ($\approx$200 to 400 mg/m^3) : Subjective complaints (fatigue or headache) but probably no observable impairment of reaction time or coordination.

>37 ppm ($\approx$150 mg/m^3) : Probably perceptible to most humans.

Because of the deficiencies in the studies on which these estimates are based, as well as variations in sensitivity to toluene that may be expected in the human population, these estimates should be regarded as approximations only.

The subchronic and chronic inhalation data lend themselves less to the definition of dose-response relationships. Most of the reports on human exposures failed to define precisely levels or durations of exposure, involved relatively small numbers of exposed individuals, and did not adequately control exposure to other toxic agents. The animal data are of little use in supporting the human data because humans appear to be more sensitive to toluene than the experimental animals on which data are available.

Qualitatively, dermal exposure to toluene can cause skin damage, as is the case with many solvents, but systemic signs of intoxication are likely to occur only in cases of gross overexposure.

1.8. RESEARCH NEEDS

Although the available data from human and animal toxicologic studies indicate that ambient exposure to toluene does not currently present a human health hazard, it is apparent that further investigation is needed in several areas. Some of the most important areas in which incomplete information is available, and that should be considered in formulating research needs, are represented below. This list of research needs, however, is not structured in terms of relative priorities among the various areas of investigation noted below.

1. Monitoring data. Up-to-date monitoring data pertaining to atmospheric levels around point sources involving solvent use, ambient air levels in rural and remote areas, and drinking water levels are needed to more accurately evaluate human exposure to toluene.

2. Consumer exposure. General population exposure to toluene from gasoline usage/spillage during vehicular filling operations, from use of paint and varnish thinners/removers, and from the use of other toluene-containing consumer products remains unevaluated, although exposure from these sources could substantially contribute to total exposure. Data are needed on the magnitude, frequency, duration, and extent of exposure(s) from these sources to properly assess general population toluene exposure.

3. Neurobehavioral toxicity. It is evident that the CNS is most sensitive to the effects of toluene. Although the effects of acute high level toluene exposure are fairly well documented, there is a paucity of data regarding the behavioral and neurological effects of pure toluene at low levels (i.e., less than 100-200 ppm) of exposure. In particular, the extent, nature (i.e., permanence) and threshold of neurobehavioral effects need to be determined to properly evaluate potential risk from ambient exposure.

4. Carcinogenicity. Although it is improbable that toluene is a strong carcinogen, the possibility that it is a weak one cannot be excluded on the basis of the information that is presently available. The results of the ongoing NTP carcinogenesis bioassays of toluene should help resolve the issue, and mitigate concern for the apparent deficiencies of the CIIT (1980) bioassay.

5. Mutagenicity. Toluene has been shown to be non-mutagenic in a variety of microbial and mammalian systems, but the results of cytogenetic assays (sister-chromatid exchange and chromosome aberration) are conflicting. Additional testing is needed to resolve the possibility that toluene may be a weak clastogen or mutagen.

6. Teratogenicity. Toluene has been reported to be teratogenic to mice following oral exposure (Nawrot and Staples, 1979), but not to mice or rats following inhalation exposure (Hudak and Ungvary, 1978; Litton Bionetics, Inc., 1978b; Tatrai et al., 1980). The uncertainty over the teratogenicity of toluene should serve as a stimulus for further research, including evaluation of the association between teratogenic effects and chronic low level exposure to toluene.

7. Reproductive effects. The reports of dysmenorrhea in female workers (Matsushita et al., 1975), degeneration of germinal epithelium in the testes of male rats (Matsushita et al., 1971), and increased follicle-stimulating hormone (FSH) levels in rats (Andersson et al., 1980) that have been associated with toluene exposure suggest that the reproductive effects of this compound should also be considered in formulating research needs. Again, this should include evaluation of effects associated with chronic low level exposures to toluene.

8. Respiratory Defense Mechanism. Various gaseous air pollutants, (e.g., nitrogen oxides and ozone) have been demonstrated to affect respiratory tract defense mechanisms, resulting in increased

susceptibility to respiratory infection in some mammalian species (e.g., rat, mouse) studied. Complex dose-response relationships have been demonstrated, whereby specific patterns of duration and frequency of exposure, as well as the concentration of the particular gaseous air pollutant, appear to be important factors. To date, little or no information has been published in regard to the evaluation of potential effects of volatile organic substances such as toluene on respiratory defense mechanisms. It is recommended that such studies be conducted in the near future.

2. INTRODUCTION

EPA's Office of Research and Development has prepared this health assessment to serve as a "source document" for Agency use. The scope of this document addresses toluene in relation to the total environment. It is expected that this document will serve the information needs of many government agencies and private groups that may be involved in decisionmaking activities related to toluene.

In the development of the assessment document, existing scientific literature has been surveyed in detail. Key studies have been evaluated and summary and conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified.

The present document represents an up-to-date evaluation of the available toluene data base. The document assesses all major sources of toluene in the environment, general ambient concentrations representing potential human exposure levels, and health effects demonstrated to be associated with exposure of man or lower organisms. More detailed, updated evaluations regarding sources, emissions, ambient air concentrations, and public exposure levels will be carried out in the future should EPA decide to undertake specific regulatory action(s) for toluene.

The information found in this document is integrated into a format designed as the basis for performing risk assessments. Where appropriate, the authors of the document have attempted to identify gaps in current knowledge that limit risk evaluation capabilities.

3. PHYSICAL AND CHEMICAL PROPERTIES

Toluene is a homolog of benzene in which one hydrogen atom has been replaced by a methyl group. Some of the relevant physical and chemical properties of toluene are described below.

3.1. SYNONYMS AND TRADE NAMES

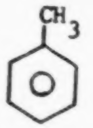
Methacide
Methylbenzene
Methylbenzol
Phenylmethane
Toluol

3.2. IDENTIFICATION NUMBERS

Chemical Abstracts Service (CAS) No.: 108-88-3

Registry of Toxic Effects of Chemical Substances (RTECS) No.: XS5250000

3.3. STRUCTURE, MOLECULAR FORMULA, AND MOLECULAR WEIGHT



Molecular Formula: C_7H_8

Molecular Weight: 92.13

3.4. PHYSICAL PROPERTIES

3.4.1. Description. Toluene is a clear, colorless liquid at ambient temperature that has a benzene-like odor. It is both volatile and flammable (Windholz, 1976).

3.4.2. Other Physical Properties.

Melting Point (Weast, 1977):	-95°C
Boiling Point (Weast, 1977):	110.6°C
Density (g/mℓ, 20°C) (Weast, 1977):	0.8669
Specific Gravity (15.6/15.6°C) (Cier, 1969):	0.8623
Vapor Pressure (25°C) (Weast, 1977):	28.7 torr
Vapor Density (air = 1) (Weast, 1977):	3.20

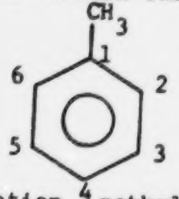
Vapor Pressure (25°C) (Weast, 1977):	28.7 torr
Vapor Density (air = 1) (Weast, 1977):	3.20
Percent in Saturated Air (760 mm, 26°C) (Walker, 1976):	3.94
Density of Saturated Air-Vapor Mixture (760 mm (air = 1), 26°C) (Walker, 1976):	1.09
Solubility (Sutton and Calder, 1975):	
Fresh water (25°C)	534.8 mg/ℓ
Sea water (25°C)	379.3 mg/ℓ
Flammable Limits (percent by volume in air) (Walker, 1976):	1.17 to 7.10
Flash Point (closed cup) (Walker, 1976):	40°F
Autoignition Temperature (Walker, 1976):	552°C
Log Octanol-Water Partition Coefficient (Tute, 1971):	2.69
Odor Threshold in Air (Walker, 1976):	
Coke derived	4.68 ppm
Petroleum derived	2.14 ppm
Surface Tension (20°C) (Walker, 1976):	28.53 dynes/cm
Liquid Viscosity (20°C) (Walker, 1976):	0.6 cp
Refractive Index (68°F) (Cier, 1969):	1.49693
Conversion Factor (in air, 25°C):	$1 \text{ ppm} \equiv 3.77 \text{ mg/m}^3$ $1 \text{ mg/m}^3 \equiv 0.265 \text{ ppm}$

3.4.3 Significance of Physical Properties with Respect to Environmental Behavior. The volatility of toluene, as indicated by its relatively high vapor pressure, indicates that a substantial fraction of environmental toluene is likely to be present in the vapor phase mixed with air. The relatively high volatility of toluene, combined with its low solubility in water, may lead to intermedia transfer of toluene from water to the air phase. The details of the environmental fate of toluene as determined by its physical and chemical properties are discussed in Chapter 6.

The log octanol-water partition coefficient for toluene may have significance in determining its affinity toward organics in soil and aquatic organisms. The details of the bioconcentration factor for toluene based on the octanol-water partition coefficient value are discussed in Chapter 9. The knowledge of physi cal properties such as flammable limits and flash point is important for the safe handling and transport of toluene; data on density and solubility may be necessary for health effect studies.

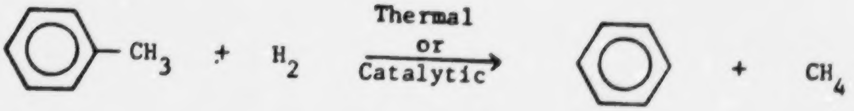
3.5. CHEMICAL PROPERTIES

Toluene undergoes substitution reactions, either on the aliphatic side group ($-CH_3$) or on the benzene ring. These substitutions occur exclusively at the ortho (2) and para (4) positions marked in the following figure:



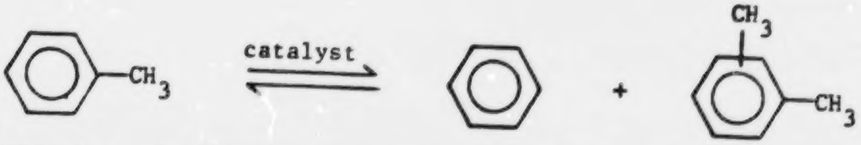
Nitration, sulfonation, halogenation, methylation, and chloromethylation are some examples of substitution reactions. These reactions occur at a rate between 2.1 and 467 times faster with toluene than with benzene (Cier, 1969).

The methyl group in toluene is susceptible to dealkylation to produce benzene (Bradsher, 1982).

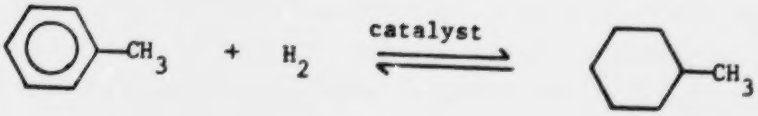


At one time, the most significant use of toluene was in the production of benzene by the above reaction (Cier, 1969).

Toluene undergoes a reversible disproportionation and transalkylation reaction in the presence of a catalyst (Cier, 1969).

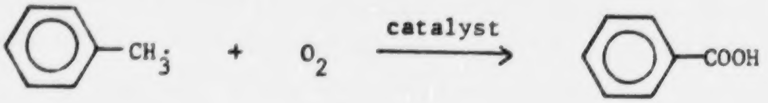


Hydrogenation of toluene takes place readily to produce methylcyclohexane (Cier, 1969).

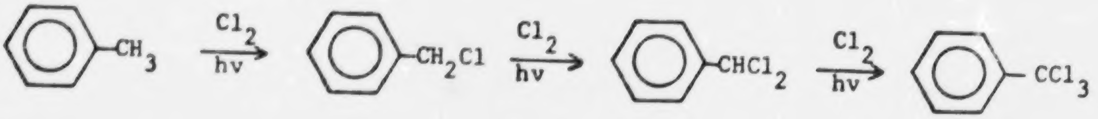


The reverse process of dehydrogenation of methylcyclohexane is the principal mode of toluene manufacture. Methylcyclohexane is found in petroleum fractions, along with other naphthenes (Cier, 1969).

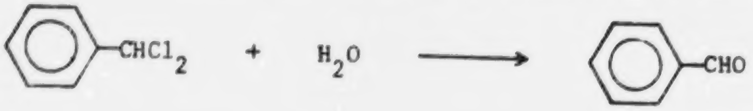
Oxidation of toluene under catalytic conditions yields benzoic acid as a principal product (Cier, 1969).



Chlorination of toluene under actinic light conditions yields methyl substitution products (Cier, 1969).

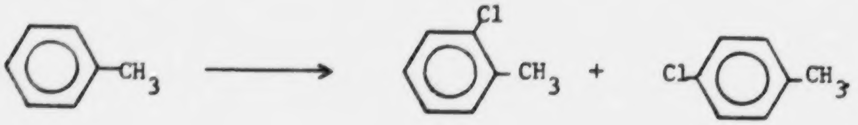


The hydrolysis of benzalchloride produces benzaldehyde (Gait, 1967).



The above reactions may have some significance with respect to chlorination of drinking water. The oxidation of toluene that occurs in drinking water may be one of the sources of benzaldehyde and benzoic acid detected in drinking water (U.S. EPA, 1980).

In the presence of catalysts and in the absence of light, chlorination produces o- and p-chlorotoluene (Cier, 1969).



In the vapor phase, toluene is relatively unreactive toward RO_2 radicals and O_3 found in the troposphere. It is, however, relatively more reactive toward OH radicals; the products of the reaction are normally benzaldehyde and cresols (Brown et al., 1975). This reaction may have significance with respect to the fate of toluene in the atmosphere and is discussed in detail in Section 6.1.

Toluene forms azeotropes with a number of solvents, including paraffinics, naphthenics, and alcoholic hydrocarbons. Azeotropes are important in the purification of toluene, in solvent technology, and in the recovery of toluene from reaction mixtures (Cier, 1969).

Toluene is marketed as nitration grade (1°, boiling range of 1°C), pure commercial grade (2°C), and all other grades. Generally accepted quality standards for the first two grades are given by the American Society for Testing and Materials (Cier, 1969). The actual concentration of toluene is not stipulated in these specifications; however, the nitration grade (1°) and pure commercial grade (2°) toluene are of 99.5 to 100% and 98.5 to 99.4% purity, respectively (USITC, 1979). All other grades include toluene and are used as solvent grade and for blending aviation and motor gasoline. The non-fuel toluene (solvent grade) is of 90 to 98.4% purity (USITC, 1979).

Commercial toluene may contain benzene as an impurity. Therefore, all health effect studies involving toluene should specify the quality of toluene used for experimentation. If benzene is present in the toluene, it must be demonstrated that the observed health effects are not wholly or partly due to benzene. Because of this contamination, it may also be necessary to determine the amount of benzene released to the environment due to industrial usage of toluene.

In general, toluene is quite stable in air, and most of the chemical reactions discussed above require specialized conditions. While some of the reactions may have environmental significance, the majority of the chemical reactions discussed above are conducted under conditions of commercial and research applications.

3.6. REFERENCES

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4. PRODUCTION, USE, AND RELEASES TO THE ENVIRONMENT

4.1. MANUFACTURING PROCESS TECHNOLOGY

Toluene is produced primarily from three sources: (1) petroleum refining processes, (2) indirectly as a by-product of styrene production, and (3) indirectly as a by-product of coke-oven operations.

4.1.1. Petroleum Refining Processes. Low levels of toluene are present in crude petroleum. Toluene is produced from petroleum by two processes: (1) catalytic reforming and (2) pyrolytic cracking.

4.1.1.1. CATALYTIC REFORMING -- The largest quantity of toluene produced in the United States is generated in the catalytic reforming process. The total estimated toluene produced in this process in 1978 was 3110 million kg. This represents about 87% of the total amount of toluene produced in the United States in 1978 (Table 4-1).

Catalytic reforming involves the catalytic dehydrogenation of selected petroleum fractions that are rich in naphthenic hydrocarbons to yield a mixture of aromatics and paraffins. The proportions of aromatics and paraffins in the reformate depend upon the feedstock used and the severity of the reforming operation (Cier, 1969). At present, reforming operations are geared primarily to producing a benzene-toluene-xylene (BTX) reformate from which the individual aromatics are recovered (Cier, 1969). Toluene is isolated from the reformate by distillation, followed by washing with sulfuric acid and redistillation. Only a small fraction of catalytic reformate, however, is utilized for isolating toluene. The unseparated toluene in catalytic reformate is used for gasoline blending.

4.1.1.2. PYROLYTIC CRACKING -- The second largest quantity of toluene comes from pyrolytic cracking. Of the total isolated toluene produced in the United States in 1978, approximately 9% (324 million kg) was obtained from this source (Table 4-1).

When heavier hydrocarbons, such as hydrocarbon condensates, naphtha, and gas oil, are pyrolytically cracked for the manufacture of olefins, pyrolysis gasoline is produced as a by-product. The amount of pyrolysis gasoline produced from pyrolytic cracking depends on the feedstock and the manufacturing conditions (Mara et al., 1979). The by-product, pyrolysis gasoline, contains a high percentage of aromatics. Toluene can be isolated from pyrolysis gasoline by

TABLE 4-1

U.S. Production of Isolated Toluene in 1978[a]

Production Process	Amount Produced (10^6 kg)	Percent of Total
Catalytic reforming	3110	86.5
Pyrolytic cracking	324	9
Styrene by-product	135	3.8
Coke oven by-product	26[b]	0.7
TOTAL	3595	100

[a] Source: Little, 1981

[b] This value does not include toluene obtained from tar distillers.

distillation, removal of any olefins and diolefins, and redistillation. Not all pyrolysis gasoline produced in the United States is used for the production of isolated toluene.

4.1.2. By-Product of Styrene Production. When styrene is produced by the dehydrogenation of ethylbenzene, some toluene is also synthesized as a by-product. The toluene isolated from the by-product is unsuitable for chemical or solvent use. Therefore, toluene obtained from this source is used either for gasoline blending or as feed for the manufacture of benzene by the hydrodealkylation process (Mara et al., 1979). In 1978, approximately 135 million kg of isolated toluene, which was about 4% of the total, was obtained as the by-product of styrene production (Table 4-1).

4.1.3. By-Product of Coke-Oven Operation. The production of coke by the high-temperature carbonization of coal yields coal-tar and crude light oil as by-products; both of these by-products contain toluene. The production of toluene from distillation of coal-tar is minimal (Mara et al., 1979); however, some toluene is isolated from crude light oil. As shown in Table 4-1, approximately 26 million kg of toluene were isolated from coal-derived toluene in the year 1978. This amounted to about 0.7% of the total isolated toluene produced during the same year.

4.2. PRODUCERS

Of the total toluene produced in the United States for internal consumption, only about 11% is isolated as toluene (Table 4-2). The remainder stays in gasoline as a benzene-toluene-xylene (BTX) mixture. The total amount of toluene available in the United States in 1978, both isolated and non-isolated, is shown in Table 4-2.

The identification of isolated toluene producers, their estimated toluene producing capacity, and the estimated amount of toluene produced in 1978 from catalytic reforming, pyrolytic cracking, and styrene by-product are shown in Tables 4-3, 4-4, and 4-5. The identification of the producers of isolated toluene from coke-oven by-product is given in Table 4-6; the capacity for isolated toluene production and the actual amount of toluene produced are not given because the data are unavailable. It should be pointed out that many producers captively consume the toluene that they produce.

During 1979, the production of toluene from coke-oven operators had a reported increase of 17.6% over 1978 (USITC, 1979). The production of toluene from petroleum refiners has been reported to have decreased by 4.3% during the

TABLE 4-2

Isolated and Non-Isolated Toluene Available in the United States in 1978[a]

Source	Quantity (10^6 kg)	
	Isolated	Non-Isolated as BTX
Catalytic reforming	3,110	27,000
Pyrolytic cracking	324	197
Styrene by-product	135	NA
Coke oven by-product	26	96
Imports	192	
Exports	-364	NR
SUBTOTAL	3,423	27,293
TOTAL	30,716	

[a] Source: Little, 1981

NA = not applicable, NR = not reported

TABLE 4-3

Producers of Isolated Toluene from Catalytic Reforming in 1978[a]

Company and Location	Toluene Capacity (10^6 kg)	Isolated Toluene Produced (10^6 kg)
Amerada Hess - St. Croix, VI	460	310
American Petrofina - Big Spring, TX	164	110
Beaumont, TX	125	84
Ashland Oil - Catlettsburg, KY	99	67
N. Tonawanda, NY	39	26
Arco - Houston, TX	125	84
Wilmington, CA	49	33
Charter Oil - Houston, TX	39	26
Coastal States - Corpus Christi, TX	56	38
Commonwealth - Penuelas, PR	395	266
Crown - Pasadena, TX	46	31
Exxon - Baytown, TX	411	277
Getty - Delaware City, DE	--[b]	NA
El Dorado, KS	20	13
Gulf - Alliance, LA	194	130
Philadelphia, PA	92	62
Port Arthur, TX	49	33
Kerr McGee - Corpus Christi, TX	148	100
Marathon - Texas City, TX	72	49
Mobil - Beaumont, TX	280	189
Monsanto - Chocolate Bayou, TX	33	22
Pennzoil - Shreveport, LA	--[c]	NA
Phillips - Sweeney, TX	33	22
Guayama, PR	335	226
Quintana-Howell - Corpus Christi, TX	56	38
Shell - Deer Park, TX	197	133

Table 4-3. (cont.)

Company and Location	Toluene Capacity (10^6 kg)	Isolated Toluene Produced (10^6 kg)
Sunoco - Corpus Christi, TX	138	93
Marcus Hook, PA	151	102
Toledo, OH	247	166
Tulsan, OK	66	44
Tenneco - Chalmette, LA	115	78
Texaco - Port Arthur, TX	92	62
Westville, NJ	132	89
Union Oil - Lemont, IL	56	38
Union Pacific - Corpus Christi, TX	99	67
TOTAL	4613	3108

[a] Source: Little, 1981

[b] 1980 capacity for this producer was 85 million kg.

[c] 1980 capacity for this producer was 72 million kg.

NA = not applicable.

TABLE 4-4

Producers of Isolated Toluene from Pyrolysis Gasoline[a]

Company and Location	Toluene Capacity (10^6 kg)	Isolated Toluene Produced (10^6 kg)
Arco - Chanelview, TX	105	76
Commonwealth - Penuelas, PR	49	36
Dow - Freeport, TX	13	9.4
Gulf - Cedar Bayou, TX	66	48
Mobil - Beaumont, TX	16	15
Monsanto - Chocolate Bayou, TX	132	96
Union Carbide - Taft, LA	66	48
TOTAL	447	328.4

[a] Source: Little, 1981

TABLE 4-5

Producers of Isolated Toluene from Styrene By-Product[a]

Company and Location	Styrene Capacity (10^6 kg)	Isolated Toluene Produced (10^6 kg)
American Hoechst - Baton Rouge, LA	400	16
Arco - Beaver Valley, PA	100	4
Cos-Mar - Carville, LA	590	24
Dow - Freeport, TX	660	26
Midland, MI	140	5.5
El Paso Natural Gas - Odessa, TX	68	2.7
Gulf - Donaldsville, LA	270	11
Monsanto - Texas City, TX	680	27
Standard Oil (Indiana) - Texas City, TX	380	15
Sunoco - Corpus Christi, TX	36	1.4
U.S. Steel - Houston, TX	54	2.2
TOTAL	3400	134.8

[a] Source: Little, 1981

TABLE 4-6

Producers of Isolated Toluene from Coke-Oven Crude Light Oils[a]

Plant	Location
Armco	Middletown, OH
Ashland Oil	Catlettsburg, KY N. Tonawanda, NY
Bethlehem Steel	Bethlehem, PA Sparrows Pt., MD
CF and I	Pueblo, CO
Interlake	Toledo, OH
Jones and Laughlin	Aliquippa, PA
Lone Star	Lone Star, PA
Republic Steel	Youngstown, OH Cleveland, OH
U.S. Steel	Clairton, PA Geneva, UT

[a] Source: Little, 1981

same period (USITC, 1979). This resulted in a net decrease of 4.2% in the overall isolated toluene production in 1979 as compared to 1978 (Table 4-1) (USITC, 1979).

4.3. USERS

As mentioned in Section 4.2., most of the toluene produced as BTX mixture is never isolated but remains in various refinery streams for use in gasoline. Isolated toluene, on the other hand, is used for different purposes; the consumption of isolated toluene in different usage is shown in Table 4-7. The fluctuating, but largest, single use of isolated toluene is in the production of benzene through the hydrodealkylation (HDA) process. The fluctuation in the use of isolated toluene exists because the HDA process is used as an effective means of balancing supply and demand for benzene (Mara et al., 1979). The U.S. producers of benzene through the HDA process, their capacity, and the amount produced are shown in Table 4-8.

The second largest use of isolated toluene is back-blending into gasoline for increasing the octane ratings. Approximately 1465 million kg of isolated toluene, representing 35.1% of 1978 consumption, was used for gasoline back-blending.

The third largest use of toluene is in solvent applications, with the major use being in the paint and coatings industry. Significant amounts also are used in adhesives, inks, pharmaceuticals, and other formulated products. With the establishment of federal and state laws limiting the emission of aromatic solvents in the workplace and in the general environment, the demand for toluene as a solvent has declined significantly (amount unspecified) since 1975 (Mara et al., 1979). Identification of specific users of toluene as a solvent is difficult because the users are too widespread.

Another major use of isolated toluene is as a raw material in the production of toluene diisocyanate (TDI), benzyl chloride, benzoic acid, xylene, and vinyl toluene. Manufacture of phenol, cresols, toluene sulfonic acids, nitrotoluenes, terephthalic acid, caprolactam, and styrene are some of the minor uses of isolated toluene (Mara et al., 1979). A small amount of isolated toluene (6.6 million kg, <1% of total) is used for the manufacture of p-cresol (Little, 1981). The latter compound is used primarily for the manufacture of the pesticide 2,6-di-tert-butyl-p-cresol (BHT). Judging from the percent of toluene used in the manufacture of BHT, its emission from this manufacturing process should be considered insignificant.

TABLE 4-7

Consumption of Isolated and Non-Isolated Toluene in Different Usages[a]

Usage	Amount Used/year (10^6 kg)	Percent of Total Use in Each Category
Non-isolated:		
Gasoline as BTX	27,293	100
Isolated:		
Benzene dealkylation	1,675	40.2
Gasoline back-blending	1,465	35.1
Solvent for paint and coatings	263	6.3
Solvent for adhesives, inks, and pharmaceuticals	132	3.2
Toluene diisocyanate	200	4.8
Xylene	98	2.4
Benzoic acid	65	1.6
Benzyl chloride	36	0.9
Vinyl toluene	25	0.6
Miscellaneous others	39	0.9
Net export	172	4.1
TOTAL	4,170	100.1

[a] Source: Little, 1981

TABLE 4-8

Consumers of Toluene for the Manufacture of Benzene by HDA Process[a]

Company and Location	Toluene Used (10^6 kg)	Benzene Production Capacity (10^6 kg)
American Petrofina - Port Arthur, TX	59	77
Big Spring, TX	103	130
Ashland Oil - Catlettsburg, KY	91	120
Coastal States - Corpus Christi, TX	156	200
Commonwealth - Penuelas, PR	298	380
Crown - Pasadena, TX	59	77
Dow - Freeport, TX	65	84
Gulf - Alliance, LA	122	160
Philadelphia, PA	52	67
Monsanto - Alvin, TX	103	130
Phillips - Guayama, PR	103	130
Quintana-Howell - Corpus Christi, TX	191	250
Shell - Odessa, TX	18	23
Sunoco - Corpus Christi, TX	52	67
Toledo, OH	163	210
Tulsa, OK	39	50
TOTAL	1674	2155

[a] Source: Anderson et al., 1980

The identification of primary users of toluene as a chemical intermediate, their production capacity, and the amount produced is shown in Tables 4-9 and 4-10. It should be pointed out that the amount of isolated toluene used in the United States in 1978 (excluding net export) was 4000 million kg according to Table 4-7. However, Table 4-2 shows that the total amount of toluene available for internal consumption during the same period (excluding net export) was only 3600 million kg. This discrepancy is due to the fact that Table 4-7 is based on data that are only estimates, and the data in Table 4-2 were obtained from the manufacturers who reported their net toluene production to the U.S. International Trade Commission.

4.4. ENVIRONMENTAL RELEASE

The three primary sources of toluene release or emission to the environment are production, usage, and inadvertent sources. In addition to these anthropogenic sources, some toluene is released into the environment from natural sources.

4.4.1. Emission from Production Sources. Toluene can be released into the environment during its production as process losses, fugitive emissions, and storage losses. Process emissions are those that originate from the reaction and distillation vents deliberately used for venting gases. Storage emissions originate from losses during loading and handling of the product used for manufacturing processes and storage of the final product. Fugitive emissions are those that have their origin in plant equipment leaks. The air emission factors used to estimate the total emission of toluene from different production sources have been obtained from Mara et al. (1979) and the values are given in Table 4-11.

Based on the emission factors indicated in Table 4-11, the amount of toluene emitted into the atmosphere from the four production sources has been estimated in Table 4-12. Atmospheric releases of toluene from each source shown in Table 4-12 are from production of both isolated and non-isolated toluene. It is assumed that the air emission is dependent only on the manufacturing process and is the same for both isolated and non-isolated toluene from the same process.

The manufacturing processes may lead also to toluene release in other media. The release of toluene in water from petroleum refineries performing catalytic reforming and pyrolytic cracking processes is assumed to be negligible because the concentration of toluene has been determined to be below the quantification limit in more than 90% of discharged water from the refineries (Little, 1981).

TABLE 4-9

Producers of Toluene Diisocyanate (TDI) in 1978[a]

Company and Location	TDI Capacity (10^6 kg)	Toluene Used (10^6 kg)
Allied Chemical - Moundsville, WV	36	20
BASF Wyandotte - Geismar, LA	45	25
Dow Chemical - Freeport, TX	45	25
Du Pont - Deepwater, NJ	32	17
Mobay Chemical - Baytown, TX	59	32
New Martinsville, WV	45	25
Olin - Astabula, OH	14	7
Lake Charles, LA	45	25
Rubicon Chemical - Geismar, LA	18	10
Union Carbide - S. Charleston, WV	25	13
TOTAL	364	199

[a] Source: Mara et al., 1979

TABLE 4-10

Other Toluene Chemical Intermediate Users in 1978[a]

Company and Location	Production Capacity (10^6 kg)	Toluene Used (10^6 kg)
Xylene Producers		
Arco - Houston, TX	89	48
Sunoco - Marcus Hook, PA	92	50
TOTAL	181	98
Benzoic Acid Producers		
Kalama - Kalama, WA	64	33
Monsanto - St. Louis, MO	5	2
Velsical - Beaumont, TX	23	12
Chattanooga, TN	27	14
Pfizer - Terre Haute, IN	3	1
Tenneco - Garfield, NJ	7	3
TOTAL	129	65
Benzyl Chloride Producers		
Monsanto - Bridgeport, NJ	36	16
Sauget, IL	36	16
Stauffer - Edison, NJ	5	3
UOP - E. Rutherford, NJ	1	0.5
TOTAL	78	35.5
Vinyl Toluene Producers		
Dow - Midland, MI	27	25

[a] Source: Mara et al., 1979

TABLE 4-11

Toluene Air Emission Factors from Production Sources[a]

Source	Emission Factor (kg lost/kg produced)			
	Process	Storage	Fugitive	Total
Catalytic reforming	0.00002	0.00006	0.00002	0.0001
Pyrolytic cracking	0.00015	0.00060	0.00015	0.0009
Styrene by-product	0.00001	0.00060	0.00015	0.00076
Coke oven by-product	0.00050	0.00060	0.00015	0.00125

[a] Source: Mara et al., 1979

TABLE 4-12

Estimated Atmospheric Toluene Emissions from Four Major Production Sources

Production Source		Total Amount Produced (million kg/yr)	Total Emission Factor	Total Emission (10^3 kg/yr)
Catalytic reforming	- Isolated	3,110		
	- Non-isolated	27,000	0.0001	3,011
Pyrolytic cracking	- Isolated	324		
	- Non-isolated	197	0.0009	469
Styrene by-product		135	0.00076	103
Coke oven by-product	- Isolated	26		
	- Non-isolated	96	0.00125	153
TOTAL				3,736

Coking operations, however, can lead to toluene release in other media. The toluene-containing wastewaters from coking plants that originate from waste ammonia liquor, final cooler blow down, and benzol plant wastes have the following distribution (Little, 1981):

Direct discharge: 33%
Publicly Owned Treatment Works (POTW): 25%
Quenching: 40%
Deep well injection: 2%

Two-thirds of the wastewater from the quenching operation is recirculated and actually not discharged. Therefore, only 73% of the total wastewater containing toluene is actually discharged to the environment.

The average volume of effluents produced from coke-oven operation (Little, 1981), the toluene concentration in these effluents (Little, 1981), and the emission factors in these effluents are given in Table 4-13.

For a total coke production of 44×10^9 kg in 1978 (Little, 1981), the total amount of toluene discharged in wastewater is calculated to be $44 \times 10^9 \times 4.43 \times 10^{-6} \times 0.73 = 142 \times 10^3$ kg. Some toluene in wastewater may finally enter other media, because wastewater from the quenching operation is sent to sumps that generate only solid and gaseous wastes (Little, 1981). Therefore, the distribution of total released toluene in untreated wastewater can be estimated as given in Table 4-14.

4.4.2. Emission from Toluene Usage. The emission of toluene from various usages has been estimated from emission factors and the amounts used. The values for the emission factors obtained from Mara et al. (1979) are shown in Table 4-15.

The atmospheric emission of toluene from its production sources, such as gasoline in non-isolated BTX and the isolated form (for back-blending), has already been included in Table 4-12. The emission factor for miscellaneous uses has been assumed to be the average of other toluene usages excluding its use as a solvent. All the toluene used in paint and coatings has been assumed to be ultimately released to the atmosphere (Mara et al., 1979). Therefore, an emission factor of 1.0 has been estimated for this usage. Fifteen percent of the toluene used as a solvent for adhesives, inks, and pharmaceuticals is recovered for fuel use (Mara et al., 1979); the remainder is emitted to the atmosphere. Hence, an emission factor of 0.85 has been assumed for this usage.

Based on the emission factors given in Table 4-15, the estimated toluene emissions from its various usages are shown in Table 4-16.

TABLE 4-13

Toluene Emission Factors in Wastewater from Coke Oven Operation[a]

Effluent	Liters of Effluent Produced/kg Coke	Toluene Conc. (mg/ℓ)	Emission Factor (kg/kg coke)
Waste ammonia liquor	0.16	3.1	0.496×10^{-6}
Final cooler blow down	0.13	17.0	2.21×10^{-6}
Benzol plant wastes	0.20	8.6	1.72×10^{-6}
TOTAL			4.43×10^{-6}

[a] Source: Little, 1981

TABLE 4-14

Toluene Released in Different Media from Coke-Oven Wastewater[a]

Medium	Percent of Total Released	Amount released/yr (10^3 kg)
Air	20	28
Water	33	47
Land	22	31
POTW	25	36

[a] Toluene releases from quenching are arbitrarily assumed to be evenly distributed between land and air.

TABLE 4-15

Toluene Emission Factors for Its Uses[a]

Usage	Emission Factor (kg lost/kg used)			
	Process	Storage	Fugitive	Total
Benzene production	0.00005	0.00010	0.00005	0.00020
Solvent for paint and coatings	NA	NA	NA	1.0
Solvent for adhesives, ink, pharmaceuticals, and others	NA	NA	NA	0.85
Toluene diisocyanate	0.00077	0.00032	0.00019	0.00128
Xylene production	0.00005	0.00010	0.00005	0.00020
Benzoic acid	0.00100	0.00040	0.00010	0.00150
Benzyl chloride	0.00055	0.00030	0.00015	0.00100
Vinyl toluene	0.00055	0.00030	0.00015	0.00100
Miscellaneous	NA	NA	NA	0.00100

[a] Source: Mara et al., 1979

NA = not applicable.

TABLE 4-16

Estimated Toluene Emission from Different Uses

Source	Amount Used/yr (10^6 kg)	Emission Factor (kg lost/kg used)	Total Emission/yr (10^3 kg)
Benzene production	1675	0.0002	335
Solvent for paint and coatings	263	1.0	263,000
Solvent for adhesives, inks, pharmaceuticals, and others	132	0.85	112,000
Toluene diisocyanate	200	0.00128	256
Xylene production	98	0.0002	20
Benzoic acid	65	0.00150	98
Benzyl chloride	36	0.0010	36
Vinyl toluene	25	0.0010	25
Miscellaneous others	39	0.0010	39
TOTAL	2533		375,809

It can be concluded from Table 4-16 that, among the different usages of toluene, the maximum emission (excluding inadvertent sources) occurs from solvent application (see Section 4.4.3.).

The released toluene from the different user sources shown in Table 4-16 has been assumed to enter only one medium, air. The use of toluene as a solvent, however, has been found to produce toluene in wastewater (Little, 1981). Table 4-17 shows the total estimated release of toluene to aqueous media from its use as a solvent in different industries.

4.4.3. Emission from Inadvertent Sources. Because gasoline consumes a vast amount of total toluene produced (Table 4-7), this use constitutes the largest source of environmental emission of toluene. The emission of toluene from its use in gasoline can occur from three distinct sources: evaporation from its use in the automobile, evaporation from marketing activities (handling and transfer of bulk quantities), and emission from automobile exhaust.

Other inadvertent sources of toluene emissions into the environment include transportation spills into surface water and land, other manufacturing processes not producing toluene, different combustion sources, and cigarette smoke (Table 4-18). The inadvertent release of toluene from other manufacturing processes occurs primarily from feedstock contamination, by-product formation, and the use of oil. An example of the latter source is in the manufacture of acrylonitrile in which wastewater ponds are covered with oil to control the release of volatile organics.

The release of toluene into different media from various inadvertent sources is shown in Table 4-18. Intermedia transfers of the compound will possibly change the emission values given in Table 4-18 because of the volatility of toluene.

4.4.4. Non-anthropogenic Sources. Substantial amounts of toluene can be released into the environment from petroleum seepage in the oceans and on land, and from the weathering of exposed coal strata. However, no estimate of total environmental release of toluene from these natural sources is available. Some vegetations can be natural sources of toluene in the environment. Toluene is naturally produced by the tropical tolu tree. It has been identified in roasted filberts, in peanuts and macadamia nuts, in grape essence, and in cooked potatoes (NRC, 1980). It is, however, unlikely that significant amounts of toluene are released into the environment from the vegetations in the United States (NRC, 1980; Seila, 1979).

TABLE 4-17

Toluene Released in Aqueous Media from Use as a Solvent in Various Industries[a]

Source	Toluene Conc. in Wastewater (μg/ℓ)	Percent Occurrence	Wastewater Discharged (10^6 ℓ/d)	Amount of Toluene Released (10^3 kg/yr)[b]
Ink formulating	1600	87	0.092	0.038
Textile products	14	46	2000	3.8
Gum and wood chemicals	2000	78	0.11	0.17
Paint formulating	990	87	2.8	0.72
Leather tanning	78	25	200	1.2
Pharmaceuticals	515	62	250	24
TOTAL				29.9

[a] Source: Little, 1981

[b] Based on 300 operating d/yr.

TABLE 4-18

Toluene Emission from Different Inadvertent Sources[a]

Source	Environmental Release (10^3 kg/yr)		
	Air	Water	Land
Gasoline marketing	19,000	NR	NR
Automobile gasoline evaporation	18,000	NR	NR
Automobile exhaust	640,000	NR	NR
Transportation spills:			
Oil	NR	400	5.6
Gasoline	NR	680	230
Toluene	NR	2.2	11
Propylene oxide manufacture	36	NR	NR
Polychloroprene manufacture	460	NR	NR
Ethylene-propylene rubber manufacture	90	NR	NR
Ethylene-propylene terpolymer production	4,200	NR	NR
Wood preserving industry	NR	6.3	NR
Insulation board manufacture	NR	neg.	NR
Hardboard manufacture	NR	neg.	NR
Acrylonitrile manufacture	59	NR	NR
Combustion processes:			
Coal refuse piles	4,400	NR	NR
Stationary fuel combustion	13,000	NR	NR
Forest fires	7,000	NR	NR
Agricultural burning	1,000	NR	NR
Structural fires	<1,000	NR	NR
Cigarette smoke	53	NR	NR
Others	8	NR	NR
TOTAL	708,306	1,089	247

[a] Source: Little, 1981

NR = not reported

4.4.5. Sum of Emissions from All Sources. The emissions of toluene into different media from all sources are given in Table 4-19. The estimates also include toluene emission from coke production. The emission of toluene from coke oven operation is based on an emission factor of 0.00024 (Mara et al., 1979) and an estimated coke production of 44 x 10^9 kg (Little, 1981) for the year 1978.

It is evident from Table 4-19 that the toluene released into the environment predominantly enters one medium, the atmosphere. The three largest sources of toluene emission in descending order are auto exhaust, solvent use, and evaporative loss from automobile and service stations. A large amount of toluene from land and water spills is also likely to enter air as a result of evaporation. The large figure for the combined release of toluene into the atmosphere explains the reason for its presence as the aromatic hydrocarbon of highest concentration in the ambient atmosphere (Chapter 7).

4.5. USE OF TOLUENE IN CONSUMER PRODUCTS

The consumer products shown in Table 4-20 and analyzed prior to 1969 may contain some toluene. The percent of toluene in these products also is indicated in the same table. The emission of toluene into the environment from this source is already included under Section 4.4.2.

Information available through the Food and Drug Administration (FDA) (Bolger, 1981) shows that of the 19,500 cosmetic products registered with the FDA through August 14, 1979, 664 products contain varying percents of toluene. One of the products contains more than 50% toluene, 166 products contain 25 to 50% toluene, 492 products contain 10 to 25% toluene, 1 product contains 1 to 5% toluene, and 4 products contain 0.1% or less toluene. The use of toluene is related to nail base coats, nail enamel, nail polish removers, and other manicure products.

TABLE 4-19

Total Yearly Release of Toluene into Different Media

Source	Environmental Release (10^3 kg/yr) Air	Water	Land	POTW
Production (see Tables 4-12 and 4-14)	3,764	47	31	36
Usage (see Tables 4-16 and 4-17)	375,809	30	NA	NA
Inadvertent (see Table 4-18)	708,306	1,089	247	NA
Coke production	10,560	NA	NA	NA
TOTAL	1,098,439	1,166	278	36

NA = not available.

TABLE 4-20

Consumer Product Formulations Containing Toluene[a]

Product	Percent Toluene Content
China cement, solvent type	20 to 30
Contact rubber cement	may contain toluene
Microfilm cement, cotton base	27 to 30
Model cement	up to 20 to 25
Plastic cement, polystyrene	24
Shoe cement	may contain toluene
Tire repair, bonding compounds	>80
Paint brush cleaners	contain 25 to 90 BTX
Stain, spot, lipstick, rust removers	may contain toluene
Nail polish	35
De-icers, fuel antifreeze	30
Fabric dyes	$\leq$60
Indelible inks	may contain toluene
Marking inks	80 to 90
Stencil inks	40 to 60
Solvents and thinners	may contain toluene

[a] Source: Gleason et al., 1969

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5. ABATEMENT PRACTICES IN INDUSTRY

The four major potential sources of toluene release to the environment, in order of importance (Table 4-19), are (1) inadvertent sources, such as vehicular emissions and losses during gasoline transfer, (2) solvent use in paint, coating adhesives, and inks, (3) coke production, and (4) manufacturing sites such as petroleum refineries and chemical plants. Therefore, the institution of pollution control devices for these four major sources can be expected to produce a large impact on the overall toluene level in the environment.

5.1. ABATEMENT PRACTICES FOR INADVERTENT SOURCES

The two major sources of vehicular emissions of toluene in the atmosphere are exhaust emissions and evaporative emissions from the gas tank and the carburator. Crankcase emissions have been eliminated essentially through the use of positive crankcase ventilation technologies (U.S. EPA, 1980).

The installation of catalytic converters on automobiles has resulted in a significant reduction of hydrocarbon emissions from automobiles. Generally, tailpipe catalysts control systems remove unsaturated and aromatic hydrocarbons, including toluene, more efficiently than paraffinic hydrocarbons (U.S. EPA, 1980). Therefore, both the photochemical reactivity and the mass of hydrocarbons emitted are reduced by the catalytic converter systems.

Evaporative emissions from automobiles have been reduced through the use of adsorption regeneration carbon canister technologies (U.S. EPA, 1980). Such systems are more effective, however, for regular grade gasoline containing 25 to 27% aromatics than for premium grade unleaded gasoline containing 43% aromatics (U.S. EPA, 1980).

Most of the current diesel exhaust emission studies are concerned with emission controls through either engine design or the use of fuel additives. Other control options, such as catalytic reactors, appear to be viable (Santodonato et al., 1978).

Other major sources of automobile emissions are losses from spilled gasoline and losses during fuel transfer. The former can be reduced by educating the public about the necessity of restricting spillage both for economic and environmental reasons. The loss of gasoline during fuel transfer is already controlled in many areas of the country by incorporating vapor recovery systems (NRC, 1980).

5.2. ABATEMENT PRACTICES FOR SOLVENT USAGE

Solvent vapors originating from industrial use of toluene in coatings and thinners can be controlled or recovered by applying condensation, compression, adsorption, or combustion principles. Control efficiencies of 90% or greater are possible by activated carbon adsorption, provided that particulates are removed from the contaminated airstream by filtration before the airstream enters the carbon bed (U.S. EPA, 1980).

When recovery of the vapor is not desired, an incineration method can be used for controlling emissions. The choice between direct flame and catalytic incineration methods must be based on economic factors and on local emission standards.

Control of toluene emissions from gravure printing can be done in a number of ways (U.S. EPA, 1980). Process modifications involving microwave, infrared, electron beam, or ultraviolet drying and subsequent recovery of organic vapors will reduce emissions. Another alternative is to replace inks containing organic solvents with aqueous or solventless inks. Incineration of the exhaust gases by thermal or catalytic methods provides another method of emission control. Last, solvent vapors can be adsorbed in activated carbon as a method of controlling toluene vapor emissions into the atmosphere.

5.3. ABATEMENT FOR COKE OVEN EMISSIONS

Hydrocarbon emissions result from the burning of the stripped coke oven gas for the under-firing of the coke batteries. The combustion exhaust gases from each oven are combined together and vented through a common stack. Improving the combustion efficiency of the coke batteries would be a proper method of control (U.S. EPA, 1980).

5.4. ABATEMENT FOR EMISSIONS FROM MANUFACTURING SITES

Current technology for the control of gaseous hydrocarbon emissions from manufacturing sites takes the form of charcoal adsorption, direct flame or catalytic incineration, chemical sorbents, vapor condensation, process and material change, and improved maintenance (U.S. EPA, 1980). The feasibility of sorbing organics by the wet scrubbing method, using selected aqueous surfactant systems as opposed to plain water, has been demonstrated (Matunas et al., 1978). Organic removal as high as 90 to 95% can be attained by using this method. Condensation of organics by the removal of heat may be an expensive method since refrigeration must be used for the removal of heat from gases (Matunar et al., 1978).

5.5. ABATEMENT PRACTICES FOR RAW AND FINISHED WATERS

No information could be found on this subject. Treating water with activated carbon, however, is expected to remove toluene from drinking waters.

5.6. ECONOMIC BENEFITS OF CONTROLLING TOLUENE EMISSIONS

There is no significant geographical area in the United States in which ambient concentrations of alkylbenzenes are known to be harmful to plants or animal lives (NRC, 1980); however, as reactive hydrocarbons, they can contribute to the formation of photo-chemical smog that is known to be harmful to life and property. Brookshire et al. (1979) selected residential properties in six pairs of selected neighborhoods and found the property value could increase on the average of $504 annually if the air quality were improved. The authors ascribed about one-half of the enhanced value to respondent-perceived aesthetic benefits (visibility) and the other half to perceived health benefits. Thayer and Schulze (1980) extrapolated the results of Brookshire et al. (1979) to the entire south coast air basin of California and concluded that the urban benefits from improved air quality amounted to between $1.6 billion and $3 billion in the basin. The benefits that an improved air quality would provide for commercial agriculture in southern California can be added to the urban benefits described above. Adams et al. (1980) examined the economic impact of ambient oxidants upon 14 selected crops in the region. They extrapolated their results of these 14 crops to all southern California commercial agricultural products and predicted a $250 million benefit to be derived from control of oxidants in the air.

All of the cost benefits discussed above are based on total pollutants in air. It is not possible to project the portion of these benefits that may be attributable to the control of toluene pollution alone. For a detailed description of the cost benefits of controlling alkylbenzene pollution, interested readers are referred to a recent NRC (1980) document.

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6. ENVIRONMENTAL FATE, TRANSPORT, AND PERSISTENCE

The environmental fate, transport, and transformation of toluene in three different media--air, water, and soil, are individually discussed below.

6.1. AIR

6.1.1. Fate in Air. Toluene can persist in the atmosphere. It is, therefore, a prime candidate for short- and long-range transport away from urban emission sources. The dispersion of toluene from a point source to the ambient atmosphere can be modeled theoretically by using dispersion equations. One such modeling method has been used in the Integrated Exposure Analysis Section (Section 10) to determine the transport characteristics of toluene.

The atmospheric toluene concentration downwind from one of the largest U.S. automobile manufacturing plants was measured by Sexton and Westburg (1980). At a point 6 km from the plant site, the toluene concentration was found to be 20.5 ppb. The concentration of toluene was still 15.1 ppb at a point 18 km downwind.

Toluene itself does not absorb light at wavelengths longer than 295 nm. The solar spectrum in the troposphere does not contain much light of wavelengths shorter than 295 nm. Therefore, toluene can absorb only insignificant amounts of sunlight in the lower atmosphere, but a charge-transfer complex between toluene and molecular oxygen absorbs light of wavelengths to at least 350 nm. According to Wei and Adelman (1969), it is the photolysis of this complex that may be responsible for some of the observed photochemical reactions of toluene.

Toluene apparently is removed from the atmosphere primarily through free radical chain processes (NRC, 1980). Of the free radicals in the atmosphere, hydroxy (•OH), atomic oxygen (O), and peroxy ($\cdot HO_2$ or $\cdot RO_2$, where R is an alkyl or acyl group) radicals are potential initiators for the removal of toluene. An additional reactive species is ozone. The rate constants for the reaction of these species with toluene and their relative significance for toluene removal are given in Table 6-1.

It is obvious from Table 6-1 that reactions with hydroxy radicals are the most important processes for the removal of toluene from the atmosphere. Based upon an estimated daytime hydroxy concentration given in Table 6-1 and a rate constant for the reaction of •OH radicals with toluene of 6.4×10^{-12} cm^3 mol^{-1} sec^{-1} (Perry et al., 1977), the chemical lifetime of toluene in daylight hours has been estimated to be 43 hours. The atmospheric residence time of toluene due

TABLE 6-1

Rate Constants for Reactions of Toluene with Reactive Species in the Atmosphere[a]

Species	Estimated Average Daytime Annual Concentration ppm	Rate Constant, ppm^{-1} min^{-1}	Rate of Toluene Removal, ppm/min	Fraction of Hydroxyl Rate
Hydroxyl radical	4×10^{-8}	9.5×10^{3}	3.7×10^{-4}	1
Atomic oxygen	3×10^{-9}	1.1×10^{2}	3.3×10^{-7}	10^{-3}
Peroxy radical	1×10^{-4}	2.5×10^{-7}	2.5×10^{-11}	4×10^{-8}
Ozone	3×10^{-2}	5×10^{-7}	1.5×10^{-8}	5×10^{-5}

[a] Source: NRC, 1980

to •OH radical reactions has been estimated to be 1.9 days by Cupitt (1980). However, the half-life or residence time value is subject to considerable uncertainty and may vary on a day-to-day basis by as much as an order of magnitude depending on solar intensity, temperature, and local trace gas composition of the atmosphere.

The reaction products formed from toluene under simulated atmospheric conditions are not known with certainty. According to the study of O'Brien et al. (1979), the gaseous products of the reaction are o-cresol, m- and p-nitrotoluene, benzyl nitrate, and benzaldehyde. Of these products, o-cresol and benzaldehyde are the major components, each composing about 8% of the total product yield. The mechanisms by which these products are formed are shown in Figure 6-1.

It is assumed that the reaction proceeds via addition of •OH radicals to the ring or by abstraction of hydrogen from the methyl side chain. Several investigators have determined the relative importance of both reaction pathways. From the amounts of reaction products formed, it was determined that the addition mechanism is of much greater significance than the abstraction mechanism (Kenley et al., 1978; O'Brien et al., 1979; Hoshino et al., 1978).

Other reaction products also are formed from toluene reactions under simulated atmospheric conditions. Some of the ring fragmentation products formed are acetylene, acetaldehyde, and acetone. The total yield of these products is much less than 1%. Formaldehyde and formic acid are also formed, but their yields are not known. A measurement of the total gas phase carbon showed that 60% of the oxidation products from the photodecomposition of toluene left the gas phase and deposited on the walls of the reaction vessel or formed an aerosol (NRC, 1980). The distribution of the products between gas and condensed phases (aerosol) in the open atmosphere (as opposed to the reaction in a vessel) is still not clear.

In addition to the above photooxidation products, photolysis of toluene in polluted atmospheres (containing NO_x) yields ozone and fairly high amounts of peroxyacetylnitrate (PAN) (5 to 30% nitrogen yield) and peroxybenzoylnitrate (PBzN) (0 to 5% nitrogen yield) (NRC, 1980). The mechanism of PAN formation is either by the fragmentation of the aromatic ring or by the secondary reactions involving products of toluene photolysis. PBzN is formed by the photooxidation of benzaldehyde produced from the photooxidation of toluene (NRC, 1980). The formation of the peroxy compounds is significant because these products are

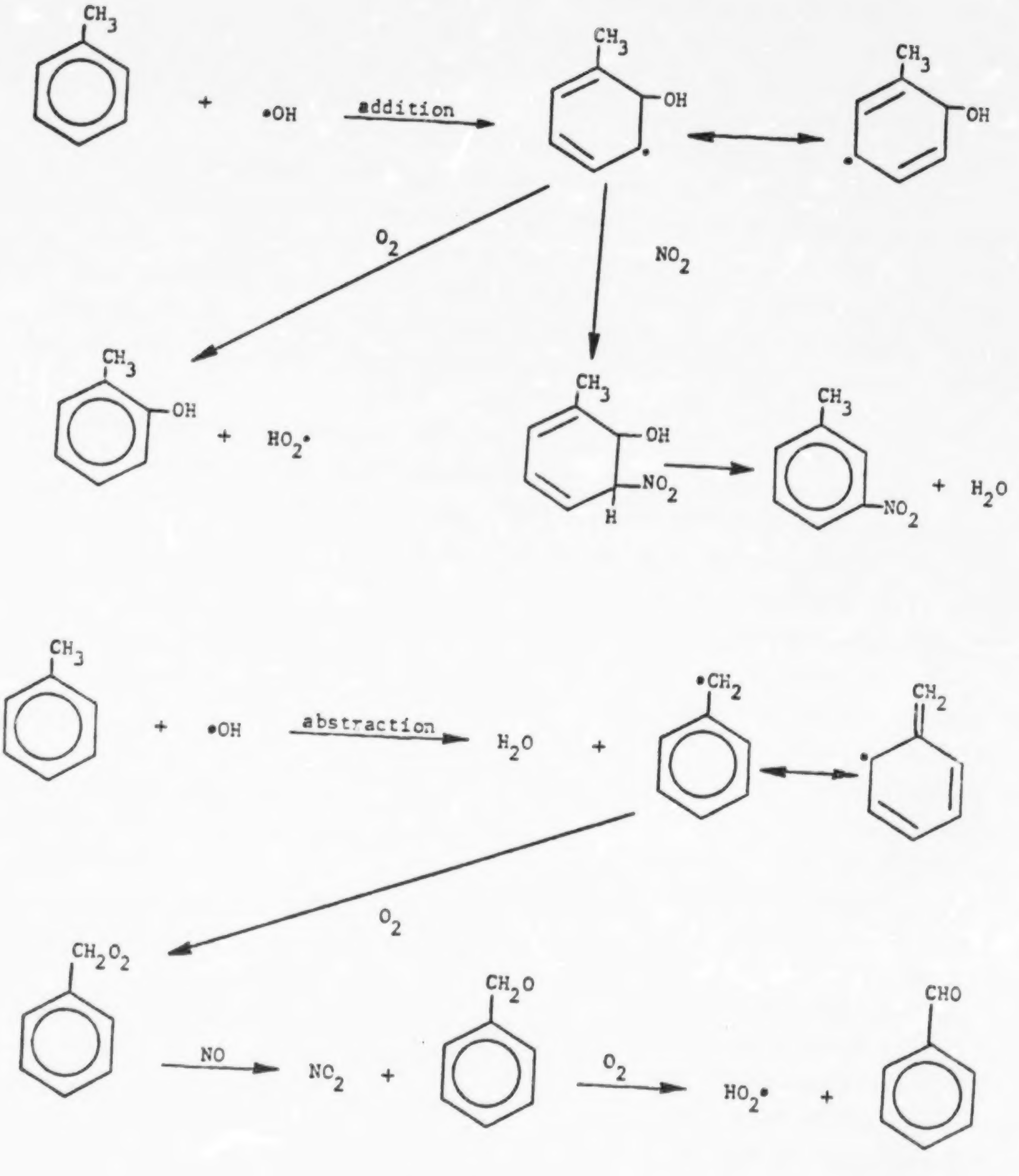

FIGURE 6-1

Proposed Reaction Pathways of Toluene Under Atmospheric Conditions

Source: NRC, 1980

strong eye irritants and oxidizing agents, and may induce plant damage (NRC, 1980). For an excellent review of the photochemical fate of toluene in the atmosphere, the reader is referred to a recent NRC document (NRC, 1980).

6.1.2. Transport. The volatility of toluene and its low solubility in water permit it to volatilize from water surfaces to the atmosphere (MacKay and Wolkoff, 1973). Studies of actual and simulated oil spills in seawater indicate that virtually all hydrocarbons smaller than C_{15} will be lost to the atmosphere within a few days (McAuliffe, 1977). The reverse process, that is, transfer of toluene from air to hydrosphere through rain, is also known to occur (Walker, 1976); however, washout should be considered an insignificant removal process for toluene from air (NRC, 1980). The probability of physical removal of toluene from the atmosphere was also speculated to be unlikely by Cupitt (1980).

6.2. AQUATIC MEDIA

6.2.1. Fate. Sauer et al. (1978) concluded from their studies of the coastal waters of the Gulf of Mexico that toluene and other alkylbenzenes are persistent in the marine environment. The probable modes of toluene loss or transformation from the aquatic environment are discussed below.

Oxidation: Reaction of toluene in water with hydroxy radicals generated from the irradiation of hydrogen peroxide produces benzaldehyde, benzyl alcohol, and cresols (Jefcoate et al., 1969). No data were found in the literature from which a relevant rate of oxidation of toluene in the aquatic environment could be determined.

It has been observed (Carlson et al., 1975) that toluene may form small amounts of chlorine-substituted products during chlorination under conditions used for water renovation. The extent of chlorination increases with the decrease of pH and increase of contact time. At a water temperature of 25°C and a chlorine concentration of 7×10^{-4} M, the percent chlorine uptake was determined to be 11.1 and 2.9% at water pH of 3 and 7, respectively (Carlson et al., 1975). With other conditions remaining the same, no chlorine uptake was observed at water pH of 10.1. Therefore, chlorination of renovated water which is usually carried out at pH levels near 7 may not be of significant environmental concern.

Hydrolysis: No data have been found that would support any role of hydrolysis in the fate of toluene in the aquatic medium.

Bioaccumulation: No measured steady-state bioconcentration factor (BCF) is available for toluene but, using the equation of Veith et al. (1979) and the measured octanol-water partition coefficient of 2.51 (U.S. EPA, 1980) (as opposed to the theoretical value for log BCF of 2.69 [Chiou et al., 1977]), the

U.S. EPA (1980) has estimated the BCF as 27.1. A factor of 3.0/7.6 = 0.395 has been used to adjust the estimated BCF from the 7.6% lipids on which the Veith et al. (1979) equation is based to the 3% lipids that is the weighted average for consumed fish and shellfish in the United States. Thus, the weighted average BCF for toluene from edible aquatic organisms consumed by Americans has been calculated to be 27.1 x 0.395 = 10.7.

In one experiment (Roubal et al., 1978), coho salmon (Oncorhynchus kisutch) and starry flounder (Platichthys stellatus) were exposed to a soluble fraction of a crude oil containing aromatic hydrocarbon in a flowing seawater. It was found that alkylated aromatics accumulated in tissues to a greater degree than unsubstituted derivatives. In both species, accumulations of substituted benzenes increased with increased alkylation. The tissues were not analyzed for toluene because of inadequate analytical procedures. It was determined, however, that the bioconcentration factors in starry flounder for C_4 and C_5 substituted benzenes were as high as 2600 and as low as near zero (concentration in fish tissue was below detection limit of 0.05 ppm) for xylenes. Substantial variations in BCF for individual hydrocarbons were found in both species. The muscle of coho salmon, which has a higher lipid content than starry flounder, showed a lower BCF. It was concluded (Roubal et al., 1978) that factors other than lipid content were more important in the observed species differences in the BCF values.

6.2.2. Transport. The primary fate-determining processes of toluene in aqueous media appear to be its intermedia transport processes (U.S. EPA, 1979). The details of the transport processes are discussed below.

Water to Air: Although there are no experimentally determined evaporation rates of toluene from water, there are theoretical models available for predicting the rate of evaporation of slightly-soluble materials from aqueous solution (Mackay and Wolkoff, 1973; Liss and Slater, 1974; Mackay and Leinonen, 1975; Dilling, 1977). The most accurate of these is based on the mass transfer coefficients for the liquid and vapor phases reported by Liss and Slater (1974) and the Henry's law constant for a solute as calculated by its solubility, vapor pressure, and molecular weight (Mackay and Leinonen, 1975). Based on these, Mackay and Leinonen (1975) reported the calculated evaporation half-life for toluene from 1m deep water to be 5.18 hours.

The intramedia transfer of toluene in water can be calculated from this half-life (t 1/2) value. If the $t_{1/2}$ and the current velocity are assumed to be

5.18 hours and 1 m/sec, respectively, the distance downstream that water in a river would flow before the volatilization of 50% toluene is:

$$5.18 \text{ hour} \times 1 \text{ m/sec} \times 3600 \text{ sec/hour} = 18,648 \text{ m}$$

Similarly, Henry's law coefficient (H) can be used to determine toluene concentration in air phase over seawater. If the height of the air and water columns are assumed to be the same, the Henry's law coefficient can be given as:

$$H = \frac{[\text{toluene}]_{\text{gas}}}{[\text{toluene}]_{\text{liq}}} = 0.349 \text{ for seawater (NRC, 1980)}$$

Thus, if equilibrium were attained, only 26% of toluene would be present in the gas phase above seawater. This calculation does not consider stratification.

In natural shallow or deep waters where stratification is expected to occur, it is likely that the atmospheric mixing layer is 10 to 100 times deeper than the aquatic mixing layer (NRC, 1980). In such water, 78 or 97%, respectively, of the toluene would exist in the gas phase.

Water to Soil: The importance of this transport process can be evaluated by experimentally determining the toluene content in sediments of surface water contaminated with toluene. Theoretical modeling can also be used for this purpose. Using the U.S. EPA's multicompartment Exposure Analysis Modeling System (EXAMS), Slimak (1980) has determined that bottom sediments account for over 90% of the total toluene discharged into surface waters under steady-state conditions. The values for the distribution of toluene between surface water and sediment as determined by the EXAMS modeling do not agree with the experimental results of Jungclaus et al. (1978). Jungclaus et al. (1978) determined the toluene content in the water and sediments of a river receiving wastewater containing toluene. Although toluene was detected in the river water, it was found not to accumulate in the sediments. More research in this area is needed to explain this discrepancy between the EXAMS modeling and the experimental results.

6.3. SOIL

6.3.1. Fate. Toluene probably exists in soils in the sorbed state. The sorption of toluene by clay minerals (bentonite and kaolinite) was studied by El-Dib et al. (1978) and was found to follow Freundlich's adsorption isotherm. These authors also found that the adsorption capacity increased as the pH value decreased.

The fate of toluene in soil has not been thoroughly investigated. It can be anticipated, however, that a portion of toluene in soil will undergo intermedia transfer to air and water, and a portion will undergo intramedia transfer. The part that stays in soil may participate in chemical reactions (including photochemical reactions) and biological degradation and transformation. The relative importance of intermedia transfer and chemical and biological reactions of toluene in soils is not known.

Investigations by Wilson et al. (1981) indicate that volatilization, biodegradation, and biotransformation processes dominate the fate of toluene in soils. The intermedia transfer of toluene from soil to water probably is not an important pathway. No data could be found in the literature searched that would support a hypothesis for any role of chemical reactions in determining the fate of toluene in soils. The intermedia transport of toluene and its biological fate in soils have been discussed separately below.

6.3.2. Transport.

6.3.2.1. SOIL TO AIR -- Laboratory experiments of Wilson et al. (1981) show that 38 to 66% of 0.2 to 0.9 mg/ℓ of toluene applied to the surface of sandy soils with 0.087% organic carbon will volatilize to air. The volatilization rate is dependent on the nature of the soil. The volatilization rate may be significantly lower for soils with high organic contents due to their sorption properties (Slimak, 1980). This phenomenon may be especially important with respect to municipal sludges that normally contain high organic substances.

6.3.2.2. SOIL TO WATER -- The transfer of toluene from soil to ground or surface waters can be of importance with regard to the possibility of contamination of these water bodies and their subsequent use as sources of drinking waters. Unfortunately, very little information is available about this subject. From the investigations of Wilson et al. (1981), it can be concluded that the transport of toluene from soil to water is probably not a major transfer pathway. These investigators showed that 2 to 13% of the applied toluene on a sandy soil system could be eluted through a column 140 cm high. The leaching of toluene from landfill sites that contain soil originated partly from municipal sludges can be expected to be even lower. The higher organic content of these soils may retard the aqueous elution process due to higher sorption properties of the soils toward toluene.

6.4. ENVIRONMENTAL PERSISTENCE

6.4.1. Biodegradation and Biotransformation

6.4.1.1. MIXED CULTURES -- The study of the disappearance of toluene in soil began nearly 75 years ago. Stormer (1908) and Wagner (1914) showed that toluene was susceptible to bacterial decomposition in the soil. Gray and Thornton (1928) and Tausson (1929) isolated soil bacteria that used toluene as a sole carbon source. Claus and Walker (1964) found that the half-life of toluene in isolated bacteria from soil inhabited with toluene-degrading bacteria was 20 to 60 minutes. Wilson et al. (1981) indicated that from 21 to 60% of toluene eluted through 140 cm of sandy soil biodegraded. The authors stated that the process was probably very sensitive to the soil type and, therefore, may or may not be an important removal process of toluene from a particular soil system.

More literature, however, exists on the biodegradation of toluene in aquatic environments. In a U.S. EPA report (Slimak, 1980), the biodegradation of toluene in lakes, rivers, and ponds was discussed using an extension of the U.S. Environmental Protection Agency's (U.S. EPA) multicompartment Exposure Analysis Modeling System (EXAMS). The report stated that the biodegradation of toluene accounted for 0.31, 4.81, 0.36, 0.09, and 18.47% of the total toluene loss in oligotrophic lakes, eutrophic lakes, clean rivers, turbid rivers, and ponds, respectively. Using the standard dilution method and filtered wastewater effluent as the seed to determine the biochemical oxygen demand (BOD), the biodegradability (percentage bio-oxidized) of toluene ranged from 63 to 86% after up to 20 days (Price et al., 1974; Bridie et al., 1979).

Matsui et al. (1975) found that in activated sludge acclimated to various organic compounds, the total organic carbon (TOC) removal efficiency for toluene was 60% while the chemical oxygen demand (COD) was 72% for 24 hours. The authors concluded, however, that although toluene was a readily biodegradable compound, in this experiment disappearance was due mainly to evaporation. Using the Warburg technique, Lutin et al. (1965) reported a 40% degradation of toluene in activated sludge after 144 hours. In comparison, 63% of the benzene was degraded in the same time. The degradation of toluene in benzene-acclimated activated sludge reached 17.2% of the theoretical BOD after 6 hours and 48% after 192 hours (Malaney and McKinney, 1966). Toluene was the most biodegradable of a number of alkylbenzenes tested by these authors, who also found that the introduction of a methyl group to benzene retarded the initial (6 hour) rate of oxidation of toluene but not the extent of degradation compared to benzene. Marion and Malaney (1964) exposed activated sludge to 500 mg/ℓ of toluene from 3 municipal plants and reported that unacclimated sludge showed little ability to oxidize benzene and toluene after 6 hours and that after 72 hours, less than 11% oxidation had taken place (compared to 44.7% reported by Malaney and McKinney, 1966). One sludge sample, however, acclimated to benzene, oxidized greater than 30% of the toluene after 180 hours. It should be noted that the high concentration of toluene used in this study probably was toxic to the organisms in the sludge.

Tabak et al. (1981) studied the biodegradation of toluene at lower concentrations (5 mg/ℓ and 10 mg/ℓ) with settled domestic wastewater as microbial inoculum by the static-culture flask-screening procedure. Toluene was completely biodegraded in 7 days at 25°C by this method. At influent concentration levels of 70 µg/ℓ and 112 µg/ℓ, the removal of toluene by activated sludge treatment at two municipal facilities was found to be at least 99% (Patterson and Kodukala, 1981). In 31 industrial facilities, activated sludge treatment was found to remove an average of 52% toluene at an average influent concentration of 119 µg/ℓ. The treatment of three industrial effluents in aerated lagoons removed an average of >93% toluene initially present at an average concentration of 143 µg/ℓ (Patterson and Kodukala, 1981).

The degradation of toluene has also been studied in mixed cultures of bacteria. Chambers et al. (1963), using phenol-adapted bacteria, reported 38% degradation of toluene after 180 minutes. It should be noted that phenol is the metabolic degradation pathway of toluene. In another study, Dechev and Damyanova (1977) grew sludge cultures in either phenol, xylene, or toluene as the sole carbon source and found that phenol-adapted bacteria proved less able to degrade xylene and toluene, while toluene-adapted cells showed greater versatility in their ability to oxidize phenol and xylene.

6.4.1.2. PURE CULTURES -- Although pure cultures do not occur in nature, a discussion of biodegradation in these media may provide insight to degradation in more complex media occurring in a natural environment. Fungi and bacteria have been shown to use toluene (Smith and Rosazza, 1974). In the course of studying the effects of toluene on microbial activity, Kaplan and Hartenstein (1979) discovered that 6 of 7 fungi imperfecti, 7 of 13 basidiomycetes, and 6 of 14 bacteria grew with 0.1 or 0.05% toluene as the sole carbon source. The addition of yeast extract increased the amount of toluene-utilizing microorganisms. In contrast, no oil-use or hydrocarbon-degrading fungi grew on toluene as the sole carbon source (Davies and Westlake, 1979). Using an oxygen electrode to measure oxidation, Buswell and Jurtshuk (1969) found that resting cells of an n-octane-utilizing Corynebacterium sp. oxidized only 7% of the available toluene compared to 100% oxidation of n-octane. Toluene did not serve as a growth substrate in this experiment. Kapraleck (1954) isolated a Pseudomonas-type bacteria from the soil of a petroleum deposit that used toluene. Pseudomonas sp. and Achromobacter sp. from soil used toluene as the sole carbon source for growth (Claus and Walker, 1964; Gibson and Yeh, 1973). Smith and Rosazza (1974) reported that bacteria and yeast hydroxylated toluene. In contrast, Nei et al. (1973) found little oxidation of toluene by phenol-using yeast.

The metabolic pathway for the bacterial oxidation of toluene has been studied with soil microorganisms (Figure 6-2) and reviewed by Gibson (1971) and Subramanian et al. (1978). On the basis of simultaneous adaptation studies, Kitagawa (1956) concluded that Pseudomonas aeruginosa oxidized toluene via benzyl alcohol and benzaldehyde to benzoic acid and then to catechol. This pathway was supported by the investigations of Nozaka and Kusunose (1968). A Mycobacterium sp. also produced benzoic acid from toluene (Atkinson and Newth, 1968), as did a methanotrophic bacterium (Methylosinus trichosporium) (Higgins et al., 1980).

An alternative pathway was proposed by Claus and Walker (1964) using a Pseudomonas sp. and an Achromobacter sp. isolated from soil that used toluene as a sole carbon source for growth. These investigators found that washed cell suspensions oxidized toluene to 3-methylcatechol, indicating that the methyl moiety was not oxidized, as occurred in the pathway proposed by Kitagawa (1956). A similar oxidation product was found by Nozaka and Kusunose (1969) using Pseudomonas mildenbergii cell-free extracts. Gibson et al. (1968a) also reported the detection of 3-methylcatechol from toluene by Pseudomonas putida. An oxidation product preceding 3-methylcatechol was found in cultures of a mutant strain of P. putida (strain 39/D) (Gibson et al., 1968b, 1970). This new product was identified as (+)-cis-2,3-dihydroxy-1-methylcyclohexa-4,6-diene (cis-2,3-dihydro-2,3-dihydroxytoluene) (cis-2,3-DH-2,3-DOH TOL) (Kobal et al., 1973). The catechol and 3-methylcatechol then can be cleaved by ortho cleavage to yield the corresponding muconic acids or by meta cleavage to yield the corresponding hydroxymuconic semialdehydes (Bayly et al., 1966). Methylmuconic acid was formed from toluene oxidation by a soil bacterium Nocardia corallina (Jamison et al., 1969). The semialdehydes are further converted to 2-hydroxy-6-oxo-2,cis-4,cis-heptadienoic acid (2-OH-6-OXO-2,cis-4,cis-HA) and then to acetate, pyruvate, and acetalydehyde and to CO_2 and energy (Bayly et al., 1966). The conversion of toluene to compounds that can be used as sources of carbon and

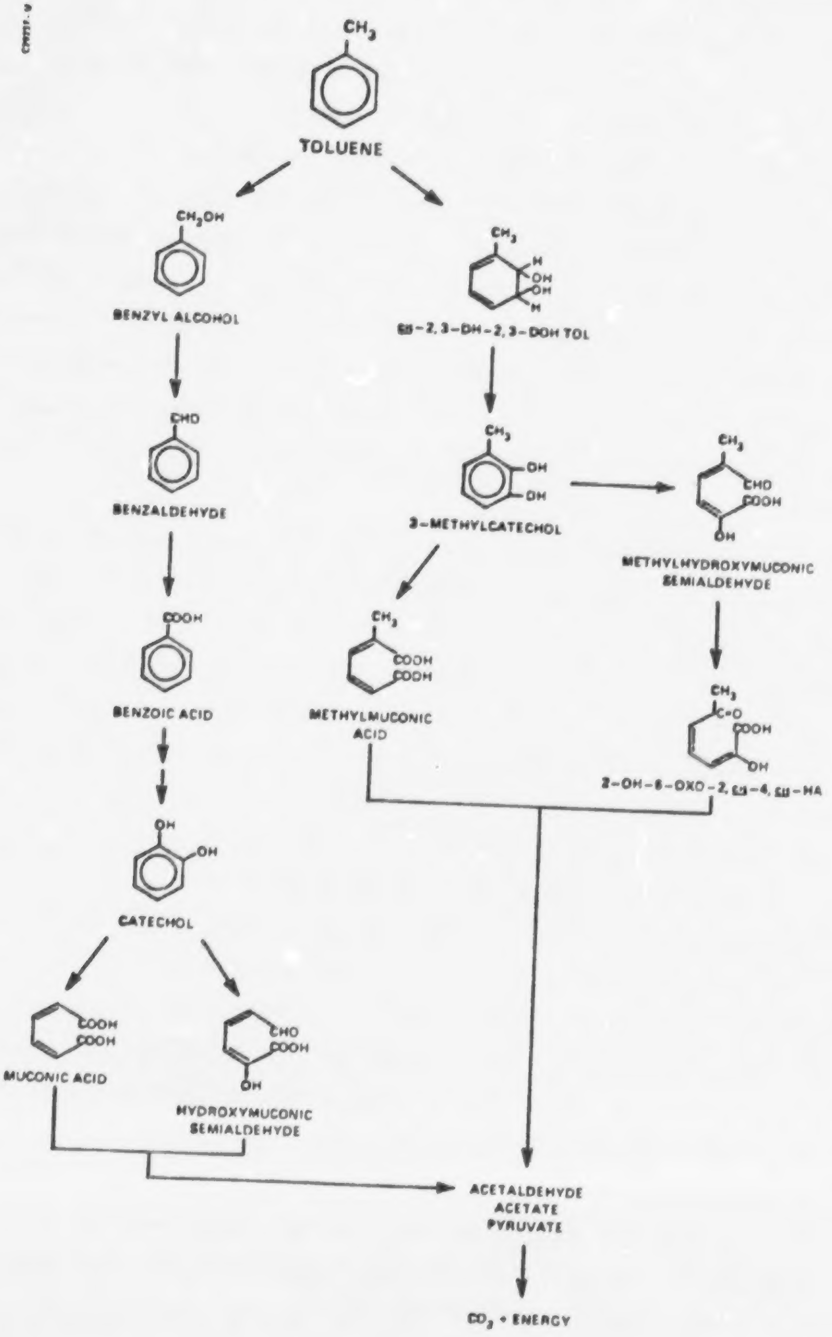

FIGURE 6-2

Microbial Metabolism of Toluene

energy suggests that toluene will be degraded rapidly by these microbial species proliferating at the expense of the compound.

The enzymes responsible for toluene degradation are carried on plasmids (Williams and Worsey, 1976; Saunders, 1977). Williams and Worsey (1976) isolated 13 bacteria from soil, all of which carried the toluene-degrading plasmids, suggesting that the plasmid-borne gene responsible for toluene degradation is wide spread in the soil microbial population. The plasmid can also be transposed into other hosts, further increasing the number of toluene-degrading bacteria (Broad et al., 1977; Jacoby et al., 1978). The toluene plasmid in Pseudomonas putida coded for the metabolism of toluene to the corresponding alcohol and aldehyde via the meta pathway, to the semialdehyde and further products (Worsey and Williams, 1975; Worsey et al., 1978). A plasmid coding for both toluene and xylene degradation in a Pseudomonas sp. has been isolated recently and characterized (Yano and Nishi, 1980). Broad et al. (1977) have speculated that the ortho pathway of toluene degradation probably is chromosomally coded.

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7. ENVIRONMENTAL AND OCCUPATIONAL CONCENTRATIONS

Monitoring data for the concentration of toluene has been divided into two subsections, one pertaining to the environmental levels and the other to the occupational levels.

7.1. ENVIRONMENTAL LEVELS

Toluene has been detected in the following environmental media: (1) air, (2) aqueous media, (3) sediments, (4) solid wastes and leachates, and (5) edible aquatic organisms.

7.1.1. Air. Toluene is the most prevalent aromatic hydrocarbon present in ambient air. Atmospheric levels of toluene in different locations in the United States and other parts of the world are given in Table 7-1. The quantification of toluene levels in the atmosphere has exclusively been done by GC-FID, especially with capillary columns (Section 8.1.1.3).

From the experimental measurements of the toluene-to-benzene ratio, Pilar and Graydon (1973) concluded that the major source of toluene in urban air with high traffic volume is automobile emission. Recently, Pellizzari (1979) has measured toluene levels near manufacturing and refining sites in the United States. The ratio of toluene to benzene in these sites indicates that besides automobile emission, manufacturing processes are probably a factor in ambient toluene concentration at many of the sites.

It can be inferred from Table 7-1 that the atmospheric concentration of toluene in urban areas not containing toluene manufacturing or gasoline refining sites are in the same range as the sites containing these industries. It can be concluded also from Table 7-1 that the concentration of toluene has declined significantly in the past 15 years in Los Angeles, presumably as a result of automotive emission controls. The concentration of toluene in many urban areas in the United States in recent years ranged from less than 0.1 ppb to as much as 50 ppb, averaging approximately 1 to 10 ppb. In remote locations of the United States, the value averaged approximately 0.3 ppb in 1971 (Table 7-1), but the current level (data reported in 1979) may be lower as indicated by the toluene concentration at Grand Canyon.

Sexton and Westberg (1980) monitored the air near an automotive painting plant at Janesville, Wisconsin, to investigate the effect of emission from paint solvents on atmospheric toluene level. The toluene concentration downwind

TABLE 7-1

Atmospheric Concentrations of Toluene

Location	Sampling date	Concentration, ppb		Reference
		Median or Average	Highest or Range	
Urban Areas:				
Azusa, CA	1967	14	23	Altshuller et al., 1971
Azusa, CA	1975	13	5.9-31	Mayrsohn et al., 1976
Baton Rouge, LA	1977	0.15	0.05-0.19	Pellizzari, 1979a
Batsto, NJ	1979	0.6	ND-3.5	Bozzelli et al., 1980
Bayonne, NJ	1969	11.8	NR	Lonneman et al., 1974
Birmingham, AL	1977	2.0	0.21-4.7	Pellizzari, 1979a
Burnett, TX	1977	30.0	NR	Oldham et al., 1979
Camden, NJ	1979	6.97	0.23-38	Bozzelli et al., 1980
Deer Park, TX	1974-1977	67	3.2-99	Oldham et al., 1979 Lonneman et al., 1979

TABLE 7-1 (cont.)

Location	Sampling date	Concentration, ppb Median or Average	Concentration, ppb Highest or Range	Reference
Denver, CO	1973	9	74	Russell, 1977
	1973-1980	8.4	0.71-37	Singh et al., 1980
				Russell, 1977
Denver City, TX	1977	1000	70-5500	Oldham et al., 1979
Edison, NJ	1976	350	NR	Pellizzari, 1977
El Dorado, AR	1978	9.7	0.12-39	Pellizzari, 1979a
Elizabeth, NJ	1978-1979	7.5	ND-85	Pellizzari, 1979a; Bozzelli et al., 1980
El Monte, CA	1975	16.0	2.9-51	Mayrsohn et al., 1976
El Paso, TX	1978	5.7	1.0-99	Pellizzari, 1979a
Houston, TX	1973-1980	10	0.21-53	Brodzinsky and Singh, 1982
Irving, TX	1977	9.5	NR	Oldham et al., 1979
Jacinto City, TX	1973-1974	18	6.3-29	Lonneman et al., 1979

TABLE 7-1 (cont.)

Location	Sampling date	Concentration, ppb Median or Average	Concentration, ppb Highest or Range	Reference
Jones State Forest, TX	1978	1.1	0.6-13.1	Seila, 1979
La Porte, TX	1973	5.6	NR	Oldham et al., 1979
Lake Charles, LA	1978	0.33	0.08-0.58	Pellizzari, 1979b
Liberty Mound, OK	1977	0.98	0.07-9.9	Eaton et al., 1979
Linden, NJ	1969	15.0	NR	Lonneman et al., 1974
Long Beach, CA	1975	6.7	1.4-23	Mayrsohn et al., 1976
Los Angeles, CA	1963-1965	59	NR	Leonard et al., 1976
	1966	37	129	Lonneman et al., 1968
	1967	30	50	Altshuller et al., 1971
	1968	39	NR	Kopeznski et al., 1972
	1971	50	NR	Altshuller et al., 1971
	1973	22	NR	Leonard et al., 1976
	1979	11.7	1.1-53.4	Singh et al., 1979

TABLE 7-1 (cont.)

Location	Sampling date	Concentration, ppb		Reference
		Median or Average	Highest or Range	
Magma, UT	1977	0.33	0.23-0.43	Pellizzari, 1979a
Manhattan, NY	1969	13.5	NR	Lonneman et al., 1974
Newark, NJ	1979	2.6	0.01-13	Bozzelli et al., 1980
Newbury Park, CA	1978	NR	0.7-13	Hester and Meyer, 1979
Oakland, CA	1979	3.1	0.15-16.9	Singh et al., 1979
Pasedena, TX	1973-1974	25	2.4-46	Lonneman et al., 1979
Philadelphia, PA	1979	4.5	2.1-5.7	Westberg and Sweany, 1980
Phoenix, AZ	1979	8.6	0.54-38.7	Singh et al., 1979
Rio Blanco County, CO	1978	1.2	0.7-2.5	Arnts and Meeks, 1981
Riverside, CA	1970-1971	NR	9-18	Stephens, 1973

TABLE 7-1 (cont.)

Location	Sampling date	Concentration, ppb Median or Average	 Highest or Range	Reference
Riverside, CA	1980	5.8	3.0-9.0	Singh et al., 1980
Rutherford, NJ	1979	8.4	0.01-33	Bozzelli et al., 1980
S. Charleston, VW	1977	0.05	0.04-0.07	Pellizzari, 1979a
South Amboy, NJ	1979	2.2	ND-9.7	Bozzelli et al., 1980
Sperry, OK	1977	1.4	0.45-4.8	Eaton et al., 1979
St. Louis, MO	1980	1.5	0.2-2.6	Singh et al., 1980
Troy, NY	NR	1.0	NR	Atwicker et al., 1977
Tulsa, OK	1977	1.6	0.04-13	Brodzinsky and Singh, 1982
Tuscaloosa, AL	1977	38	24-85	Holzer et al., 1977
Upland, CA	1975-1977	9.9	0.8-38	Brodzinsky and Singh, 1982
Vera, OK	1977	0.81	0.26-1.8	Eaton et al., 1979

TABLE 7-1 (cont.)

Location	Sampling date	Concentration, ppb		Reference
		Median or Average	Highest or Range	
Wyona, OK	1977	0.30	0.09–0.7	Eaton et al., 1979
Rural and Remote Areas:				
Brethway-Gunderson Hill, WA	1971	0.01	NR	Robinson et al., 1973
Camel's Hump, VT	1971	1.0	NR	Robinson et al., 1973
Hell's Canyon, ID	1971	0.3	NR	Robinson et al., 1973
Moscow Mt., ID	1971	0.2	NR	Robinson et al., 1973
Point Reyes, CA	1971	0.2	NR	Robinson et al., 1973
Grand Canyon, AZ	1977	Trace	Trace	Pellizzari, 1979a
Talladega National Forest, AL	1977	0.4	0.2-1.3	Holzer et al., 1977
Smokey Mountain, TN	1978	0.96	0.3-2.4	Arnts and Meeks, 1981

TABLE 7-1 (cont.)

Location	Sampling date	Concentration, ppb		Reference
		Median or Average	Highest or Range	
Global:				
Zurich, Switzerland	NR	39	NR	Grob and Grob, 1971
Toronto, Canada	1971	30	188	Pilar and Graydon, 1973
Berlin, W. Germany	1975-1976	27	2.4-94.2	Lahmann et al., 1977
Stockholm, Sweden	NR	NR	0-2.7	Johansson, 1978
The Hague, Netherland	1974	18	54	Leonard et al., 1976
Helsinki, Finland	1979	NR	15.9-37.1	Häsänen et al., 1981
Gatwick Airport, England	1979	58.6	1.2-809.6	Tsani-Bazaca et al., 1982

ND = Not Detected
NR = Not Reported

within 1.6 km of the plant was 160 ppb. The concentration of toluene was still 20.5, 22.9, 17.5, and 15.1 ppb at distances 6, 10.5, 13.5, and 16.5 km, respectively, downwind from the plant. These concentrations are about 10 to 15 times higher than the background toluene concentrations of 1.5 ppb determined at a distance of 1.6 km upwind of the plant. These concentrations are also comparable to or higher than most of the values given in Table 7-1.

In response to numerous complaints from residents about illness and odors in the vicinity of a chemical solvent reclamation plant in Maryland, Smoyer et al. (1971) monitored the valley air surrounding the plant. A toluene concentration as high as 11 ppm was registered in the valley air. Both this result and the more recent investigation of Sexton and Westberg (1980) indicate that processes involving solvent use of toluene may result in high emission of toluene in the vicinity of these sources.

7.1.2. Aqueous Media. Toluene has been monitored in a number of aquatic media including: (1) surface waters, (2) industrial wastewater, (3) water from publicly-owned treatment works (POTW), (4) underground waters, (5) municipal drinking waters, and (6) rainwater. The toluene levels in each of the media have been discussed separately.

7.1.2.1. SURFACE WATERS -- Information regarding toluene levels in surface water has been obtained primarily from the STORET system as reported by Little (1981). Table 7-2 shows the toluene levels for major river basins in the United States. It is evident from Table 7-2 that 83% of all the monitored surface water contains toluene levels below a concentration of 10 ppb. The concentration of toluene in surface waters of the central region (Lake Erie, upper Mississipi, Lake Michigan, etc.) are higher than surface waters from other regions. This higher level of toluene cannot be attributed to the emission from production sites since the central region contains only 8 of the 38 major production sites. Surface waters from Texas, which contain 20 of the 38 production sites, showed lower levels of toluene. This indicates that production processes may not be the major source of toluene emission in surface waters.

Recent studies of the coastal waters of the Gulf of Mexico have shown that aromatic hydrocarbons comprise 80 to 90% of the total dissolved volatile hydrocarbons ($<C_{14}$) at most sampling sites (Sauer et al., 1978). The volatile hydrocarbons, however, were only a few percent of the total dissolved hydrocarbons. The concentration of toluene in surface waters at several sites in the Gulf of Mexico ranged from 4.5 to 376.0 ppt, while the average was 61.4 ppt.

TABLE 7-2

Distribution of U.S. Surface Waters Within a Certain Toluene Concentration Range[a]

	Number of Observations	Percent Sample in the Toluene Concentration Range, ppb			
		<10	10.1-100	100.1-1000	>1000
Northeast	1		100		
North Atlantic	14	100			
Southeast	110	93	4	4	
Tennessee River	16	81	6	6	6
Ohio River	54	98		2	
Lake Erie	2		100		
Upper Mississippi	18	67	22	11	
Lake Michigan	30	20	77	3	
Missouri River	34	44	53	3	
Lower Mississippi	8	88		13	
Colorado River	3	100			
Western Gulf	15	100			
Pacific Northwest	80	99	1		
California	5	100			
Great Basin	1	100			
Puerto Rico	1	100			
Unlabeled	1	100			
TOTAL	393	83	14	3	IA

[a] Source: U.S. EPA, 1980a

IA = insignificant amount.

7.1.2.2. INDUSTRIAL WASTEWATERS -- Table 7-3 shows the levels of toluene in industrial effluents as stored in the STORET system (Little, 1981). It can be concluded from Table 7-3 that 85% of the effluents showed toluene concentrations of less than 10 ppb. Fifteen of the reporting stations showed toluene concentration in excess of 100 ppb.

Wastewaters from a speciality chemicals manufacturing plant were analyzed by Jungclaus et al. (1978). The concentration of toluene in the wastewater was reported to be in the range of 13 to 20 ppm. Similarly, wastewater from one tire manufacturing company was analyzed by Jungclaus et al. (1976) and was found to contain approximately 10 ppm of toluene. Both of these values are among the highest values reported in Table 7-3.

An analysis of raw wastewater and secondary effluent from a textile manufacturing plant was reported to contain toluene as one of the predominant compounds (Rawlings and Samfield, 1979). The toluene concentrations in 22 wastewater samples and 22 secondary treated effluent samples were in the range of 0.5 to 300 ppb (Rawlings and Samfield, 1979). Effluents from a paper mill in Hiro Bay, Japan, were analyzed for organic matter. It was determined that toluene constituted 1% of the total chloroform extractables from the effluent (Yamaoka and Tanimoto, 1977).

Toluene has also been detected in a variety of industrial wastewaters. Table 7-4 shows the frequency of toluene detection in industrial wastewaters (U.S. EPA, 1980a).

7.1.2.3. PUBLICLY-OWNED TREATMENT WORKS (POTW) -- A pilot study of two POTWs, one handling more organic pollutant than the other, was conducted for the U.S. EPA (1979). Toluene was detected in 100% of the influent samples and 95% of the final effluent samples from the plant containing more organic pollutants. The maximum and median toluene concentrations in the influent sample from this plant were 440 and 13 ppb, respectively. The influent sample at the other plant had maximum and median toluene concentrations of 37 and 10 ppb, respectively. The frequency of toluene occurrence at this plant was 76% for the influent and 71% for the final effluent sample.

The state of Ohio (U.S. EPA, 1977) conducted a survey of toxic substances in 2 municipal wastewater treatment plants. The toluene concentration in the wastewater of the plant dealing primarily with domestic wastewater ranged between 1 and 5 ppb. The treated effluent from the same plant, on the other hand, showed a concentration of 1 ppb. About 87% of the influent from the other plant which

TABLE 7-3

Percent Distribution of U.S. Wastewaters Within a Certain Toluene Concentration Range[a]

Effluent Discharged	Number of Observations	Percent Sample in the Toluene Concentration Range, ppb			
		<10	10.1-100	100.1-1000	>1000
Northeast	103	84	9	4	3
North Atlantic	48	88	6	6	
Southeast	100	87	10	3	
Tennessee River	28	96	4		
Ohio River	70	84	11	3	1
Upper Mississippi	64	69	30	2	
Lake Michigan	6	100			
Missouri River	16	100			
Colorado River	1	100			
Western Gulf	1	100			
Pacific Northwest	45	91	7	2	
TOTAL	482	85	11	3	1

[a] Source: U.S. EPA, 1980a

TABLE 7-4

Detection Frequency of Toluene in Industrial Wastewaters[a]

Industry	Frequency of Detection (No. Found/No. Samples)
Soap and Detergents	1/20
Adhesives and Sealants	2/11
Leather Tanning	19/81
Textile Products	56/121
Gum and Wood Products	14/18
Pulp and Paper	4/98
Timber	58/285
Printing and Publishing	50/109
Paint and Ink	48/94
Pesticides	23/147
Pharmaceuticals	38/95
Organics and Plastics	306/723
Rubber	15/67
Coal Mining	53/249
Ore Mining	6/72
Steam Electric Power Plants	32/84
Petroleum Refining	18/76
Iron and Steel	43/431
Foundries	2/54
Electroplating	5/18
Nonferrous Metals	21/173
Coil Coating	2/12
Photographic	9/25
Inorganic Chemical	10/107
Electrical	1/35
Auto and Other Laundries	9/56
Phosphates	1/33
Plastic Processing	1/1
Procelain Enameling	2/19
Landfill	3/17
Mechanical Products	23/35
Pubicly-Owned Treatment Works	11/40

[a] Source: U.S. EPA, 1980a

treated industrial-domestic wastewater showed the presence of toluene in the concentration range of 8 to 150 ppb. The frequency of toluene detection in the treated effluent from the same plant amounted to 36%. The toluene concentrations in these treated effluents ranged from 1 to 10 ppb.

7.1.2.4. UNDERGROUND WATER -- The New York State Department of Health and the United States Geological Survey examined 39 wells in 1978 for organic contamination in groundwater (Little, 1981). Toluene was detected in 85% of the wells tested. However, the toluene concentration in these waters was below 10 ppb.

Toluene concentration in well water can be obtained from the data recorded in STORET (U.S. EPA, 1980a). Eighty seven percent of the monitored data showed less than 5 ppb (detection limit) toluene. Of the 143 monitored data, only 3 indicated the presence of toluene in the concentration range of 42 to 100 ppb. All of these 3 wells were in the vicinity of landfill sites.

7.1.2.5. DRINKING WATER -- Toluene has been detected in raw water and in finished water supplies of several communities in the United States. Levels of up to 11 ppb were found in finished water from the New Orleans area (U.S. EPA, 1975a). In a nationwide survey of water supplies from 10 cities, 6 were discovered to be contaminated with toluene (U.S. EPA, 1975b). Concentrations of 0.1 and 0.7 ppb were measured in 2 of these water supplies and were detected but not quantified in the rest. Toluene was detected but not quantified in 1 of 111 finished drinking waters during a second nationwide survey (U.S. EPA, 1977). In a subsequent phase of this survey, toluene was found in 1 raw water and 3 finished waters out of 11 supplies surveyed (U.S. EPA, 1977). A level of 19 ppb measured by gas chromatography/mass spectrometry was found in 1 of these finished waters, and 0.5 ppb was found in another.

In a survey of volatile organic compounds in water at 30 Canadian potable water treatment facilities, Otsun et al. (1982) detected toluene in the raw water with a frequency of 15% and in the treated water with a frequency of 20% during the months of August and September. The average and maximum concentrations of toluene in treated Canadian water were reported to be 2 μg/ℓ and 27 μg/ℓ, respectively. The corresponding values for the raw water were <1 μg/ℓ and 15 μg/ℓ, respectively. The frequency of occurrence and the concentration of toluene in water showed seasonal variation, with the summertime values found to be higher than the wintertime values.

Nineteen volatile organic compounds, including toluene, were detected at concentrations below 5 ppb in District of Columbia drinking water (Saunders et al., 1975). These investigators also found that the concentrations of the various contaminants in tap water varied by unspecified amounts from week to week, but the chemical composition remained the same.

7.1.2.6. RAINWATER -- Toluene has been detected in rainwater from Berlin, West Germany (Lahmann et al., 1977). The toluene content in the rainwater varied with sample collection points. The rainwater from a residential area, an airport, and a busy traffic intersection showed toluene concentration of 0.13 ppb, 0.70 ppb, and 0.25 ppb, respectively.

7.1.3. Sediment. Toluene concentrations in sediment samples as recorded in STORET (U.S. EPA, 1980a) show that 91% of the samples contain less than 10 ppb of toluene. The concentration of toluene exceeded 500 ppb in only 7% of the samples. Samples with higher concentrations (greater than 500 ppb) of toluene were obtained from the vicinity of an industrial area in San Francisco.

Jungclaus et al. (1978) monitored the sediment from a river receiving industrial effluent from a specialty chemicals manufacturing plant containing toluene. However, these investigators could not detect the presence of toluene in the river sediment.

7.1.4. Edible Aquatic Organisms. Of the 59 monitored tissue samples that were recorded in the STORET system (U.S. EPA, 1980a), 95% of the data showed toluene concentrations of less than 1 ppm. The maximum toluene concentration detected in 1 fish tissue was 35 ppm. It could not be determined whether these concentrations were determined in edible flesh or in whole fish. Toluene was also detected in fish caught from polluted waters in the proximity of petroleum and petrochemical plants in Japan (Ogata and Miyaki, 1973). A concentration of 5 ppm was measured in the muscle of 1 such fish.

7.1.5. Solid Wastes and Leachates. Toluene has been detected in the air samples at a few landfill sites (U.S. EPA, 1980b) and in well water near a few landfill sites (U.S. EPA, 1980c). However, no data regarding the level of toluene in solid wastes and their leachates could be found in the literature.

7.2. OCCUPATIONAL CONCENTRATIONS

Several reports describing the presence of toluene in occupational atmospheres were found in the literature. A toluene level of 10,000 to 30,000 ppm was reported in a merchant ship after it was internally sprayed with a toluene-containing insecticide (Longley et al., 1967). Two hours after the initial monitoring, concentrations ranging from 5000 to 10,000 ppm were still present in the atmosphere of the ship.

A monitoring program was instituted in response to a report of an epidemic solvent poisoning in a rotogravure plant in Milan, Italy. Solvent containing toluene was largely used in this plant as an ink solvent and diluent. The results of the monitoring showed that the concentration of toluene ranged from 0 to 277 ppm in different parts of the work areas (Forni et al., 1971). The determined toluene concentrations at different parts of the plant during the period 1957 to 1965 are shown in Table 7-5.

TABLE 7-5

Toluene Concentrations in Different Work Areas of a Rotogravure Plant in Milan, Italy[a]

Work Area	Toluene Concentration, ppm	
	Range	Annual Mean
Center of Room	140-239	203
Folding Machines	56-277	203
Between Machine Elements	306-824	431

[a] Source: Forni et al., 1971

In 1966, the above rotogravure plant was moved to a different location and the ventilation system of the plant was improved. Subsequent analysis for toluene showed annual mean concentrations at 156 and 265 ppm near the folding machines and between the machine elements, respectively (Forni et al., 1971).

Toluene exposure levels for other occupational groups are shown in Table 7-6. Many of the levels given in this table either originate from exposure evaluation in foreign countries, or the data may be too old to have relevance in contemporary working atmospheres. Accidental exposures to toluene are discussed in Sections 11.1.1.1. and 11.1.1.2.1.

A study of 8 Japanese factories operating polychromic rotory processes for photogravure printing reported toluene concentrations in the range of 4 to 240 ppm in different work areas of the plants (Ikeda and Ohtsuji, 1969).

Toluene exposures to an unspecified number of workers in 11 leather-finishing and rubber-coating plants have also been reported (Pagnotto and Lieberman, 1967). Toluene is used as a lacquer thinner and stain remover in the leather finishing industry. In rubber-coating plants, the major source of

TABLE 7-6

Toluene Exposure Levels for Different Occupational Groups

Exposure Level	Type of Occupation	Reference
100-1100 ppm[a]	Airplane painting	Greenburg et al., 1942
150-1900 ppm[b]	Paint and pharmaceutical industry	Parmeggiani and Sassi, 1954
3-214 ppm	Automobile painting	Ogata et al., 1971
30.6 ppm (mean)[c]	Automobile painting	Hanninen et al., 1976
80-300 ppm	V-belt manufacturing	Capellini and Alessio, 1971
15-200 ppm (mean)[d]	Shoemakers	Matsushita et al., 1975
50-1500 ppm	Not stated	Wilson, 1943
200-400 ppm	Rotogravure printing	Banfer, 1961
300-430 ppm	Rotogravure printing	Munchinger, 1963
200-400 ppm	Rotogravure printing	Suhr, 1975
18-500 ppm	Rotogravure pringing	Szadkowski et al., 1976
56-824 ppm	Rotogravure printing	Forni et al., 1971
100-200 ppm (TWA) occasional rises to 500-700 ppm	Rotogravure printing	Funes-Craviota et al., 1977
16-164 ppm	Rotogravure printing	Ovrum et al., 1978
21-187 ppm	Rotogravure printing	Vaulemans et al., 1979
7-112 ppm (TWA)	Rotogravure printing	Maki-Paakkanen et al., 1980

[a] Paint contaminated with other volatile components (Table 11-9)

[b] Concomitant exposure to butyl acetate (Section 11.2.1)

[c] Concomitant exposure to other organic solvents (Table 11-3)

[d] Concomitant exposure to 20-50 ppm (mean gasoline in a few working places (Section 11.1.2)

toluene emission is the fabric-spreading machine areas. The concentration of toluene in work areas of these industries is shown in Table 7-7.

TABLE 7-7

Toluene Concentrations in Work Areas of Leather Finishing and Rubber Coating Plants[a]

Industry	Work Areas	Toluene Concentration, ppm	
		Range	Average
Leather Finishing	Finishing Area	19-85	53
	Washing and Topping Area	29-195	112
Rubber Coating	Spreading Machines	34-120	73

[a] Source: Pagnotto and Lieberman, 1967

Toluene has been detected in other occupational atmospheres. For example, a toluene concentration of 0.18 ppm has been reported in a submarine atmosphere (Chiantella et al., 1966). The origin of toluene in this atmosphere has been speculated to be paint solvents and diesel fuel used in the submarine. Toluene has been detected in the atmosphere of M15 and M19 antitank mines (Jenkins et al., 1973). The origin of toluene in this atmosphere was attributed to mine casings.

A more recent study (Fraser and Rappaport, 1976) designed to determine the health effects associated with the curing of synthetic rubber simulated the vulcanization process in the laboratory. Toluene emission in the vulcanization area from this experiment amounted to 1.1 ppm. The actual field survey of different work areas of 10 large tire manufacturing plants across the United States was conducted by Van Ert et al. (1980). The toluene concentrations in different work areas measured by these investigators is shown in Table 7-8.

It can be concluded from Table 7-8 that the extrusion process area and the tire building process area are the two areas of tire manufacturing plants that account for the major toluene emissions from these plants.

TABLE 7-8

Toluene Concentrations in Selected Work Areas of Tire Manufacturing Plants[a,b]

Work Area	No. of Plants Surveyed	Area Toluene Concentration, ppm	
		Mean	Range
Cement Mixing	8	2.9	0.2-7.7
Extrusion	4	14.0	3.3-50.0
Tire Building	2	8.0	2.5-13.4
Curing Preparation	3	0.6	0.1-1.1
Inspection and Repair	3	1.9	0.6-2.7
Warehouse	2	0.28	0.01-0.76

[a] Source: Van Ert et al., 1980

[b] All of the plants, with the exception of plants where the warehouse samples were taken, were surveyed during 1973-77. The warehouse samples were collected in 1977.

7.3. CIGARETTE SMOKE

The concentration of toluene in inhaled cigarette smoke is approximately 0.1 mg/cigarette (NRC, 1980; Dalhamn et al., 1968). Jermini et al. (1976) determined the concentration of toluene in the sidestream smoke of cigarettes. When 30 cigarettes were inhaled in a 30 m^3 room and the concentration of toluene in room air was determined, it was found to be 0.23 ppm. This value corresponds to 0.87 mg of toluene in the sidestream smoke of each cigarette. Holzer et al. (1976) determined the toluene concentration in a 60 m^3 room and found an ambient toluene concentration of 40 ppb. When 1 cigarette was smoked in the room, the concentration of toluene rose to 45 ppb. This corresponds to 1.1 mg of toluene contribution from each cigarette. It seems from this discussion that the main-stream smoke of 1 cigarette contributes 0.10 mg toluene to the smoker. The sidestream smoke, on the other hand, may contain a higher amount of toluene.

7.4. REFERENCES

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8. ANALYTICAL METHODOLOGY

Toluene has been analyzed in a number of media including the following: (1) air, (2) waters, (3) soils and sediments, (4) crude oil and organic solvents, (5) biological samples, (6) some foods, and (7) cigarette smoke. The analytical methods for the determination of toluene in each of these media are individually discussed below.

8.1. AIR

In addition to the analysis of test mixtures of toluene in air for the evaluation of methods, toluene has also been determined in ambient air, occupational air, forensic air, and air containing the pyrolysis products of organic wastes.

8.1.1. Ambient Air. The determination of toluene in ambient air consists of two distinct steps: sampling and analysis.

8.1.1.1. SAMPLING -- Toluene can be collected from ambient air in several different ways including grab sampling in aluminized plastic bags (Neligan et al., 1965), Tedlar bags (Altshuller et al., 1971; Lonneman et al., 1968), and glass containers (Schneider et al., 1978; Pilar and Graydon, 1973). Although the grab sampling is conceptually the simplest approach, this collection method without subsequent concentrative technique does not provide sufficient quantity of toluene for analytical detection and quantification. Since ambient samples contain toluene in the parts per billion range, preconcentration steps are often necessary.

Sample collection by cryogenic procedures (Seifert and Ullrich, 1978) is an alternative method for the collection of toluene in ambient air; however, the drawbacks of this procedure include the inconveniences in sampling and sample regeneration. Also, unless the moisture in air is removed, it condenses in the collection tube and may reduce or restrict the air flow through the collection tubes. Various drying agents, such as anhydrone, anhydrous K_2CO_3, ascarite, LiH, and molecular sieve can be used. It has, however, been demonstrated by Isidorov et al. (1977) that it is impossible to find a drying agent that will preferentially absorb the moisture from air without absorbing some of the trace organics.

Reversible sorption on various high surface area materials provides an excellent method for preconcentrative collection of toluene from ambient air. Since the moisture content in the air is normally 3 to 4 orders of magnitude higher than the total organics (Isidorov et al., 1977), the chosen sorbents must show little affinity toward moisture. Otherwise, the retention capacity of the sorbents will be reached much sooner than desired.

A number of sorbents such as Tenax GC (Holzer et al., 1977; Krost et al., 1982), various carbonaceous materials (Burghardt and Jeltes, 1975; Holzer et al., 1977; Isidorov et al., 1977), Polisorbimid (Isidorov et al., 1977), molecular sieves and spherisil (Ball, 1976), and Porapak Q (Johansson, 1978) have been successfully used. Typically, sampling is performed by drawing air through a trap containing the selected sorbent with battery-operated diaphragm pumps. The air flow through the trap is controlled by needle valves and measured by a previously calibrated rotometer. The trap is kept at ambient temperature to avoid condensation of water. At the end of the sampling, the trap-ends are closed with caps and transferred to the laboratory in a refrigerated state, to avoid sample loss.

8.1.1.2. ANALYSIS -- The method of analysis usually depends on the method of sample collection. The earlier investigators who used plastic bags or glass bottles for collection of grab samples used a trapping system for concentrating a relatively large volume (1 to 10 ℓ) of sample before analysis. In this method, the collected sample is allowed to flow through a cryogenic trap containing suitable sorbents. At the end of trapping, the coolant is removed from the trap and the trap is heated quickly to vaporize and transfer the trapped compounds into the gas chromatographic (GC) columns. The columns used by earlier investigators (Lonneman et al., 1968; Altshuller et al., 1971) for aromatic separations consisted of long open-tubular columns coated with m-bis(m-phenoxy-phenoxy)-benzene combined with Apiezon grease on a packed dual column with SF-96 as the liquid phase (Pilar and Graydon, 1973).

The more recent methods, which use sorbents for trapping organics, connect the trap to a GC system via multiple-port gas sampling valves. The trap is heated quickly and the desorbed organics are passed through the chromatographic columns. Because the collected samples contain a multitude of organics, capillary columns are normally used for the resolution of the organics. The Grob and Grob (1971) technique, involving the passage of the thermally desorbed organics through a small uncoated section of the capillary column cooled cryogenically, is used. When the collection is completed, this section of the capillary is heated quickly and the sample is separated on the remaining portion of the analytical column. A number of coating materials for capillary columns including

Emulphor ON-870 (Holzer et al., 1977), UCON 50 HB 2000 or 5100 (Johansson, 1978), dinonyl phthalate (Isidorov et al., 1977), Al_2O_3 (Schneider et al., 1978), DC-550 (Louw and Richards, 1975), OV-17 and OV-101 (Pellizzari et al., 1976) have been used.

In one method, thermal desorption of the organics from the sorbents was replaced by solvent desorption (Burghardt and Jeltes, 1975). In this procedure, the organics sorbed on activated carbon were desorbed by CS_2. A part of the CS_2 was injected into a packed column GC containing a long column coated with 1,2,3-tri-(2'-cyanoethoxy)-propane.

The quantification of toluene separated by the GC columns is done almost exclusively by flame ionization detectors (FID). Confirmation of the authenticity of the GC peaks often is provided by coupled mass spectrometers (MS), with or without the aid of a computerized data system (Holzer et al., 1977; Pellizzari et al., 1976; Krost et al., 1982).

A continuous automated procedure for determining toluene in the ambient air was developed by Hester and Meyer (1979). This method needs no sample preconcentration prior to analysis. In this method, a small diaphragm pump activated by a timer automatically injects air into a 1 mℓ gas-sampling (GS) loop of a GC every 10 minutes. The separating column was packed with Chromosorb P coated with N,N-bis(2-cyanoethyl)formamide. Since no concentration method was employed, the detector used had about two orders of magnitude higher sensitivity than flame ionization detectors. A photo-ionization detector was found to show the required sensitivity.

8.1.1.3. PREFERRED METHOD -- The preferred method for the monitoring of toluene in ambient air consists of sorbent collection, thermal elution, and GC-FID determination. Collection by trapping toluene in a solid sorbent provides a concentration method during sample collection. Thermal desorption is preferred over solvent elution because of the higher sensitivity of the former method. Tenax GC is perhaps the most suitable sorbent for sample collection. The collection and thermal desorption efficiency of toluene is excellent with Tenax GC. The generation of artifacts during thermal elution with Tenax GC can be eliminated largely by proper clean-up of the sorbent, and by following the GC conditioning procedure (Holzer et al., 1977). The greatest advantage of the ambient sorption-thermal elution method is its extreme simplicity and speed.

The separation and quantification of sorbent desorbed components can be achieved by means of the GC-FID method. Although photo-ionization detectors

(PID) may have higher sensitivity than flame ionization detectors, this higher level of sensitivity is not required when the samples are preconcentrated by solid sorbents. High resolution capillary columns are a necessity because of the observed complexity and low concentration of the samples. Of the different coating materials, N,N-bis-(2-cyanoethyl)formamide and 1,2,3-tris(2-cyanoethoxy)-propane are probably most suitable for the separation of aromatic components.

8.1.1.4. DETECTION LIMITS -- The detection limit of toluene in ambient air depends on the volume of air passed through the sorbent trap. For a 25 ℓ sample, the detection limit is less than 0.1 ppb (Holzer et al., 1977) with a capillary column and flame ionization detector. When direct injection (1 mℓ) and the GC-PID method are used, the detection limit for toluene is 0.3 ppb (Hester and Meyer, 1979).

8.1.2. Occupational Air.

8.1.2.1. SAMPLING -- The concentration of toluene in occupational air normally is much higher than in ambient air. Therefore, the collection of samples in certain instances may not require a concentration step. The collection of samples by the grab method has been used by a number of authors (Tokunaga et al., 1974; Chovin and Lebbe, 1967).

Some of the earlier methods used liquid scrubbers for absorbing toluene from occupational air. A number of scrubbers, including potassium iodate in dilute sulfuric acid (Ministry of Labour, 1966), cooled organic solvents such as ethyl cellusolve acetate, dimethylformamide, and dimethyl sulfoxide in dimethyl formamide (Ogata et al., 1975), and nitrating solution (Chovin and Lebbe, 1967) have been used. In addition to the inherent limitations in its ability to overcome the interferences, this method is not convenient for the collection of breathing zone samples.

The more recent methods used solid sorbents for the collection of toluene. Silica gel (Ogata et al., 1975; Tokunaga et al., 1974), activated carbon (Esposito and Jacobs, 1977; Fracchia et al., 1977; Reid and Halpin, 1968; Fraser and Rappaport, 1976; NIOSH, 1977) and Tenax GC (Nimmo and Fishburn, 1977) are some of the sorbents used for this purpose. Aromatic hydrocarbons such as toluene are easily displaced from silica gel by water vapor, resulting in possible losses of toluene in humid atmospheres (NRC, 1980). Therefore, both activated carbon and Tenax GC are the two most frequently used sorbents for the collection of toluene from occupational air. The suitability of either of the sorbents is dictated by the method of sample analysis. When thermal desorption is used, Tenax GC is the preferred sorbent. On the other hand, activated carbon is preferred when solvent desorption is the method used.

8.1.2.2. ANALYSIS -- For grab samples, direct injections into a GC system via syringes or gas sampling loops have been applied (Tokunaga et al., 1974; Chovin and Lebbe, 1967). The separating columns used in these cases were packed columns with stationary liquid phases of either dioctyl phthalate (Tokunaga et al., 1974) or bis-(beta-cyanoethyl)formamide (Chovin and Lebbe, 1967). Flame ionization detectors were used for the quantification of toluene in both cases; however, this method is capable of analyzing toluene in work atmosphere at concentrations of around 10 ppm (Chovin and Lebbe, 1967).

Toluene collected by scrubber methods is usually analyzed by colorimetric methods. Despite variations, most colorimetric methods show interferences from other chemically similar compounds (e.g., benzene, xylenes, ethylbenzenes) that are normally co-contaminants of toluene.

The first step in the analysis of toluene collected in solid sorbents is desorption. Two methods are usually available for desorption: thermal and solvent. Carbon disulfide is the most frequently used solvent for the desorption of toluene from solid sorbents (Esposito and Jacobs, 1977; Fracchia et al., 1977; Reid and Halpin, 1968; NIOSH, 1977; Van Ert et al., 1980), although some investigators have used other solvents (Ogata et al., 1975). Solvent desorption is the method of choice when activated carbon is used as the sorbent. Activated carbon has not only high efficiency of reversible toluene sorption, but it has almost quantitative toluene desorption efficiency with CS_2 (Fracchia et al., 1977). In the presence of other common organic solvents found in the work atmosphere (e.g., n-butanol, cellosolve acetate, butyl cellosolve, etc.), the CS_2 extraction efficiency decreases slightly, but addition of 5% methanol to CS_2 increases the desorption efficiency to almost quantitative value (Fracchia et al., 1977).

When Tenax GC or Chromosorb 102 is used as the sorbent, elution by thermal process is the method of choice (Nimmo and Fishburn, 1977). Although this method may require multiport sampling valves and a cryogenic sample trap for the transfer of samples from the sorbent trap to the GC system, it has certain advantages not available to solvent elution. Because the thermal desorption method uses the whole sample for quantification, it has a higher degree of sensitivity than the solvent elution method where only a small fraction of the total sample is used for quantification.

The quantification of toluene eluted from solid sorbents almost always is done by the GC-FID method. A number of packed GC columns have been used for this purpose. Dioctyl phthalate (Tokunaga et al., 1974), UCC W-982 (Nimmo and Fishburn, 1977), N,N-bis(2-cyanoethyl)formamide (Esposito and Jacobs, 1977), dinonyl phthalate (Ogata et al., 1975), and Porapak Q (NIOSH, 1977) are some of the liquid phases used for chromatographic separations.

Other methods of analysis, such as high pressure liquid chromatography (HPLC) on a reverse phase column with methanol-water as the mobile phase and ultraviolet (UV) detection, have been attempted (Esposito and Jacobs, 1977), but the sensitivity of detection was poor.

Methods involving the use of detection tubes have been applied for the determination of toluene in occupational air (Tokunaga et al., 1974). The accuracy of the detector tubes for toluene quantification is rather poor, particularly in the presence of other organic vapor (Tokunaga et al., 1974). Therefore, the detector tubes are suitable for the rough estimation of toluene concentration in the work atmosphere. More recently, detector tubes designed for long-term sampling and determination of toluene have become available. A laboratory evaluation of a few commercially available long-term detector tubes was made by Jentzsch and Fraser (1981). The results indicate that the color development of these tubes is more dependent upon humidity and sampling volume than the short-term tubes.

A simple directly-combined GC-IR (infrared) system was developed to detect low molecular weight hydrocarbons in air (Louw and Richards, 1975). In this method, the grab sample is injected directly into a GC and the effluent from the GC column is split in a certain ratio (1:49). The major portion of the effluent is directed toward a cold trap (-50°C) to freeze the organics. At the end of the trapping process, the trap is quickly heated and the released gases are allowed to pass through a microlight pipe gas cell of an IR detector. This method has been claimed to detect 14 to 19 µg of each sample component present in air (Louw and Richards, 1975); however, no field samples have been analyzed with this system.

8.1.2.3. PREFERRED METHOD -- The preferred method for monitoring toluene in occupational air can be either the NIOSH (1977) method of activated carbon sorption and CS_2 desorption or Tenax GC sorption and thermal desorption. The quantification of desorbed toluene by GC-FID is still the method of choice. As in the case of ambient air samples, N,N-bis(2-cyanoethyl)formamide liquid phase will provide one of the best separations for the aromatics.

8.1.2.4. DETECTION LIMIT -- The detection limit for toluene by carbon sorption-CS_2 desorption method depends on the volume of air sampled. Concentrations as low as 0.1 ppm toluene in a rubber tire manufacturing factory have been detected by this method (Van Ert et al, 1980). For a 100 mℓ sample, the Tenax GC sorption-thermal desorption method showed a detection limit of 0.5 ppb (Nimmo and Fishburn, 1977).

8.1.3. Forensic Air. In suspected arson cases, the method of Twibell and Home (1977) can be applied to speculate or even confirm the cause of fire. According to this method, nickel wires (curie point 358°C) coated with finely-divided activated carbon with the aid of an inert adhesive (cement binder LQ/S6), are suspended in the atmosphere under test for 1 to 2 hours at room temperature. The apparatus is connected to a GC-FID system, and the wires are heated by induction heating. The resulting chromatographic profile obtained from the desorbed gases can be compared with different fire accelerant residues (e.g., gasoline). Although the method is not quantitative, it has been claimed to show a better sensitivity than the method of hot headspace analysis (Twibell and Home, 1977)and has potential for use in cases where the presence of toluene needs confirmation, such as gasoline spills.

8.1.4. Gaseous Products from Pyrolysis of Organic Wastes. The gaseous products from a pilot plant burning such organic wastes as wood shavings, solid municipal wastes, and rice hull were analyzed by Brodowski et al. (1976). The method consisted of collecting grab samples in stainless steel sampling bulbs and injecting 0.5 mℓ of the gas into a GC. The separating columns were dual stainless steel columns packed with Porapak QS modified with terephthalic acid. Evidently, the method does not have high sensitivity of detection. The toluene concentration of the pilot plant gaseous products was determined to be 0.2 to 0.3 mol % by this method (Brodowski et al., 1976).

8.2. WATER

Toluene has been determined in a number of aqueous media including surface waters, industrial wastewaters, water from publicly-owned treatment works (POTW), underground water, drinking water, and rainwater.

8.2.1. Sampling. Water samples other than industrial wastewater samples are generally collected by the grab method. In the case of industrial discharges where the discharge parameters are dependent on the operating process, continu ous samples using a commercial composite sampler have been used (Rawlings and Samfield, 1979). The preservation and handling of the aqueous samples after collection are especially important for volatile components. The samples are collected in glass bottles that are filled to overflow and sealed with teflon-backed silicon rubber septa and screw caps. It has been demonstrated that simple samples in non-reactive matrix (e.g., drinking water, ground water) collected in the above fashion can be held under ambient conditions from 10 to 22 days without significant loss of volatile compounds (Bellar and Lichtenberg, 1979); however, wastewater samples should be adjusted to a pH of 2 by adding dilute hydrochloric acid. Any free chlorine should be neutralized by the addition of 35 mg of sodium thiosulfate per 1 ppm of free chlorine (Federal Register, 1979) before the samples are collected in glass bottles. The samples must be iced or refrigerated during transportation and storage. All such wastewater samples should be analyzed within 7 days of collection (Federal Register, 1979).

8.2.2. Analysis. Although direct injection (Jungclaus et al., 1978) and solvent extraction (Jungclaus et al., 1976) methods have been used to determine the concentration of organics including toluene in industrial wastewaters, these methods are not suitable for toluene determination in other media. Even in wastewater, both of these methods have questionable accuracy. The direct aqueous injection method does not have good sensitivity and the solvent extraction method is likely to provide low recovery since some of the volatile components will be lost during the concentrative evaporating step.

The three most commonly used methods for toluene analysis in aqueous media are (1) purge and trap, (2) headspace, and (3) sorption on solid sorbents. Each of these methods is individually discussed below.

8.2.2.1. PURGE AND TRAP -- Purge and trap is the most widely used method for the analysis of toluene in aqueous media. It has been used for the determination of toluene in drinking waters (Bertsch et al., 1975; Lingg et al., 1977; Ryan and Fritz, 1978), in wastewaters (Bellar and Lichtenberg, 1979; Rawlings and Samfield, 1979; Jungclaus et al., 1978), and in rainwater (Seifert and Ullrich, 1978). The U.S. Environmental Protection Agency recommends the use of this method for toluene analysis in wastewater (Federal Register, 1979).

In this method, an inert gas (helium) is bubbled through a water sample via a glass frit contained in a specially designed purging chamber. The aromatics released into the vapor phase are swept through and trapped in a sorbent tube. After the purging and trapping is completed, the trap is transferred to the injection port of a GC. The trap is heated and backflushed into a GC system, where the separation of the volatiles takes place. Both packed (Bellar et al., 1979; Lingg et al., 1977; Federal Register, 1979) and capillary columns (Dowty et al., 1979; Bertsch et al., 1975) employing a variety of liquid phases have been used. The resolution of components can be expected to be better with capillary columns.

The detection of the GC column effluents can be done either by flame ionization detector (Dowty et al., 1979) or photo-ionization detector (Federal Register, 1979). The use of photo-ionization detector will provide better selectivity and sensitivity of detection. The confirmation of GC peaks is usually provided by mass spectrometry aided by a computerized data system (Lingg et al., 1977; Dowty et al., 1979; Bellar et al., 1979).

A number of variations of the purge-trap method (Grob and Zurcher, 1976; Lingg et al., 1977; Dowty et al., 1979; Bellar et al., 1979) involving the variation of water volume, the temperature of the purging system, the stripping rate, the duration of stripping, the nature of sorbent, and the method of desorption (thermal versus solvent) are available. Using a 5 mℓ sample size and flame ionization detection, Dowty et al. (1979) determined the lower detection limit for toluene to be 0.1 ppb by this method. The detection limit can be further lowered if a larger volume of sample (Lingg et al., 1977) or photo-ionization detection method is used. The purge-trap method is the preferred method for monitoring toluene in both drinking and wastewater samples.

8.2.2.2. HEADSPACE ANALYSIS -- This method has not been applied frequently for the analysis of field samples; however, the method was standardized with water samples spiked with model compounds (Vitenberg et al., 1977; Drozd et al., 1978).

In the method of Drozd et al. (1978), a known volume (50 mℓ) of water is introduced into a specially designed enclosed glass apparatus (100 mℓ), and the system is thermostatically maintained at 40°C. After the system attains equilibrium (30 minutes), a known volume of headspace vapor is introduced into a capillary GC system via a trapping system consisting of a short, cooled (-70°C) precolumn coated with OV-101 (Grob and Grob technique). The separating column is coated with squalene.

The method of headspace analysis in the past had faced problems owing to the difficulty in establishing a calibration procedure. The partition coefficient of a component between gas and liquid phases is dependent on the total ionic strength in solution. Therefore, the same concentrations of a component present in two aqueous solutions of different ionic strengths but otherwise identical conditions, will not produce the same equilibrium vapor pressure. This problem of a calibration curve has been largely obviated through the development of a standard addition method (Drozd et al., 1978). Water samples containing toluene in the parts per billion range can be quantified by this method (Drozd et al., 1978) with a reasonable accuracy; however, the method may not be applicable for drinking water samples where the concentration may be lower than 1 ppb.

8.2.2.3. SORPTION ON SOLID SORBENTS -- This method is rarely used for the monitoring of toluene in aqueous samples. The applicability of the method was explored by Pfaender (1976), and Ryan and Fritz (1978) used the method for monitoring toluene in drinking water.

The method consists of passing a known volume of water through a sorbent such as XAD-2 (Pfaender, 1976) or XAD-4 (Ryan and Fritz, 1978). The sorbed organics including toluene are desorbed either by solvent extraction (Pfaender, 1976) or by thermal desorption (Ryan and Fritz, 1978), and are injected onto a GC-FID system for component separation and quantification. In the thermal desorption method of Ryan and Fritz (1978), the use of a trap consisting of a Tenax GC precolumn to eliminate the excess water showed a good sensitivity for the method. The recovery of toluene was nearly 90% when the concentration in drinking water ranged from 1 to 10 ppb. For the quantification of toluene in water by this method, the recovery of toluene from the sorbent should be known.

8.3. SOILS AND SEDIMENTS

8.3.1. Sampling. Bottom sediment samples can be collected either by Hopper-dredge or by clam-type dredge samplers (U.S. EPA, 1979). Hopper-dredge collected samples generally contain more water than clam-type dredge-collected samples. Bottom sediment samples also can be collected using a core sampler (U.S. EPA, 1979).

For volatile organic analysis, the samples should be collected in screw-capped glass containers lined with aluminum foil (Jungclaus et al., 1978) or in glass hypovials with crimped aluminum seals and teflon-backed septa (U.S. EPA, 1979). For best results, the container should be filled to maximum capacity to reduce the amount of headspace and should be transported and stored at wet ice temperature (U.S. EPA, 1979).

The method of soil sampling is given in detail by de Vera et al. (1980). The soil samples should be taken in a grid pattern over the entire site. A scoop can be used for collection of soil samples up to 8 cm deep. To sample beyond this depth, a soil auger or Veihmeyer soil sampler, as described by de Vera et al. (1980), should be used. After the sample is transferred into glass containers to a maximum capacity, the containers must be tightly capped with contamination-free lids to prevent loss of volatile components and to exclude possible oxidation. The samples should be refrigerated (4°C) during transport and storage.

8.3.2. Analysis. Very few reliable methods are available for the analysis of volatile organics in soil and sediment samples. Solvent extraction methods using highly volatile solvents are unlikely to be successful. The evaporative concentration step of this method would result in the loss of volatile organics. Headspace analysis, which has few provisions to concentrate the organics, will produce unreasonably high detection limits.

A modification of the purge and trap method has been suggested by the U.S. EPA (1979) for the analysis of soil and sediment samples. The modified purge and trap apparatus used for this purpose is described by the U.S. EPA (1979). The sample, contained in a specially-designed glass vial, is heated at 80°C and purged with helium gas. The desorbed organics are trapped in a Tenax GC column. At the end of trapping, the Tenax GC column is inserted in the injection port of a GC, and the thermally desorbed organics are analyzed by GC-FID as in the case of water and wastewater samples. The recovery of toluene was determined to vary between 32 and 44% when 0.1 to 3.0 µg of toluene was spiked onto a specially prepared soil matrix. Although the recoveries were low, they were found to be linear and reproducible (U.S. EPA, 1979). Data on spiked environmental samples showed much higher recoveries (80 to 100%).

With the purge-trap system described, the minimum detection limit of 0.1 ppb can be attained. Thus, the method showed at least two orders of magnitude higher sensitivity than headspace analysis (U.S. EPA, 1979).

8.4. CRUDE OIL AND ORGANIC SOLVENTS

Benzene and toluene concentration in petroleum crude and other fossil fuel samples can be determined by a method developed by Grizzle and Thomson (1982). In this method, the acidic and basic constituents were removed by ion-exchange chromatography prior to fractionation into groups. Alumina, chemically bonded silica-R $(NH_2)_2$, and 2,4-dinitroanilinopropyl-silica (DNAP-silica) were used for liquid chromatography class separation of aromatic hydrocarbons. On the basis of retention strengths and grouping tendencies, the DNAP-silica was found to be superior than alumina and silica-R $(NH_2)_2$.

A combination of liquid chromatography (silica gel column) and GC-FID method was employed by Fett et al. (1968) routinely to determine toluene in hydrocarbon solvents.

8.5. BIOLOGICAL SAMPLES

Toluene or its metabolites have been determined in blood, in urine, and in mothers' milk samples. These methods of analysis are discussed below.

8.5.1. Blood. Toluene in blood has been determined by GC analysis of headspace samples (Premel-Cabie et al., 1974; Anthony et al., 1978; Radzikowska-Kintzi and Jakubowski, 1981). According to this method, blood is equilibriated with air in a closed container at a fixed temperature. The headspace gas is injected into a GC-FID system for detection of toluene. The method can be used for quantification of toluene in blood by the standard addition method as described in Section 8.2.2.2.

8.5.2. Urine. In the body, toluene is mainly oxidized to benzoic acid which, after conjugation with glycine, is eliminated as hippuric acid in the urine. Hippuric acid may be formed from other metabolic processes besides toluene metabolism.

Hippuric acid in urine can be determined by a number of methods including colorimetry (Umberger and Fioresse, 1963), UV spectrometry (Pagnatto and Lieberman, 1967), and thin-layer chromatography (Bieniek and Wilczok, 1981); however, one of the better methods of hippuric acid analysis in urine was developed by Caperos and Fernandez (1977). According to this method, the hippuric acid in acidified urine is extracted with ethyl acetate. The extracted hippuric acid is esterfied with 1-p-tolyltriazene. The dried ester is dissolved in chloroform and quantified by GC-FID. The recovery of hippuric acid by this method is determined from the recovery of an added internal standard. The sensitivity of the method with 0.5 mℓ urine was determined to be 5 ppm.

Hippuric acid is an endogenous metabolite common in human urine, but toluene exposure enhances its level. However, o-cresol may be a more specific urine metabolite and may be regarded as a better index of toluene exposure in humans (Hansen and Dossing, 1982). A recent method (Hansen and Dossing, 1982) determined the urinary hippuric acid and o-cresol levels by a high-performance liquid chromatographic (HPLC) method. In this method, the hippuric acid level in urine was determined by extracting it with acetonitrile and injecting the extract onto the HPLC column. The o-cresol level in urine was determined by digesting the urine with concentrated sulfuric acid and extracting the digest with cyclohexane. The cyhclohexane layer was first washed with a phosphate buffer and finally extracted with sodium hydroxide. The sodium hydroxide phase was injected onto the HPLC column for the determination of o-cresol level. The HPLC system in both cases consisted of a Lichrosorb Si 60 column and a UV detector. The detection limits were found to 0.05 mg/mℓ and 0.05 µg/mℓ for urine hippuric acid and o-cresol, respectively.

8.5.3. Mother's Milk. The levels of toluene in mother's milk for populations in the vicinity of chemical manufacuring plants and/or industrial user facilities in the United States were measured by Pellizzari et al. (1982). The volatile compounds including toluene in the milk samples were determined by the purge and trap method (Section 8.2.2.1.), followed by thermal desorption and capillary GC-MS analysis. Of the total of 12 samples collected, 8 samples qualitatively showed detectable levels of toluene. The detection limit for these analyses was not specified by the authors.

8.6. FOODS

A headspace GC technique for quantification and a GC-MS technique for confirmation were used to determine trace amounts of toluene in plastic containers (Hollifield et al., 1980). The sample, taken in a specially enclosed vial, was heated at 90°C for 2 hours, and 2 mℓ of headspace gas was injected into a GC system. The principle of standard addition was used for the quantification of toluene. Toluene present in parts per billion range can be determined by this method.

8.7. CIGARETTE SMOKE

The concentration of toluene both in sidestream smoke (Jermini et al., 1976) and mainstream smoke (Dalhamn et al., 1968a) has been determined. For the determination of toluene in mainstream smoke, standard cigarettes were smoked by machine under standardized conditions (a 2 second 35 mℓ puff once every minute). The mainstream smoke is collected in a cold trap (Dalhamn et al., 1968b). The contents of the cold trap can be introduced into the GC by multiport valves and analyzed by GC-FID for toluene determination.

Toluene determination in sidestream smoke can be accomplished by adopting the sampling and analysis technique of Holzer et al. (1976). The sidestream smoke can be collected by drawing the smoke through a solid sorbent tube packed with Tenax GC. The Tenax GC sorbent tube can be thermally eluted onto a glass capillary column for the determination of toluene content. Adoption of a cold trap for splitless injection of the sample into the capillary column (Grob and Grob technique) will enhance the sensitivity and accuracy of the method. Additional confirmation of the GC peaks can be done by interfacing the GC with a MS (Holzer et al., 1976).

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9. EXPOSED POPULATIONS

The number of people exposed to various sources of toluene can be divided into three categories, general population, occupational group, and cigarette smokers. The breakdown of general population subjected to inhalation exposure of toluene from various sources of emissions can be obtained by performing a population analysis around each source. A computer program was used by Anderson et al. (1980) to extract site-specific population patterns from the U.S. Census figures standardized to 1978 population levels. The number of general population exposed to various levels of toluene from different sources of emission as calculated by Anderson et al. (1980) is shown in Table 9-1. For an explanation of the breakdown of the source variety shown in Table 9-1, see Section 10.1.1.

The exposed population count shown in Table 9-1 is derived from the geographical coordinate of each location. Error in the geographical coordinates of a source and population center will cause errors in population count. In addition, the population count figures obtained from the U.S. Census Bureau are subject to undercounting. The result of this undercounting will be lower population exposure estimates than the actual case.

No estimate of the number of general population exposed to toluene from ingestion of foods and drinking waters can be given. Toluene has been detected in only a small fraction of total drinking water supplies and foods that have been monitored. The number of people consuming the contaminated waters and foods is not known at the present time.

The three most likely sources that may lead to dermal exposure of toluene to the general population are usage of vehicular fuels, toluene-containing solvents, and cosmetic products containing toluene. With the recent increase of self-service gasoline stations around the country, the number of people who may inadvertently spill gasoline on parts of their body during filling operations must have increased by a large number. The deliberate use of solvents for cleaning body grease or inadvertent spillage of cleaning solvents and paint thinners on parts of the body will also lead to dermal exposure to toluene. Although the extent of exposure may be much less significant compared to the two aforementioned sources, users of cosmetic products containing toluene are another group of the general population that is exposed to toluene through the dermal route. However, no estimate is available on the number of the general

TABLE 9-1

Population Distribution and Inhalation Exposure Levels of Toluene from Different Sources[a]

Concentration Level	Number of People Exposed From: Specific Point Sources	Prototype Point Sources	Area Sources
>100	0	159	58,347
100 - >50	0	2,841	446,793
50 - >25	34	10,200	12,348,504
25 - >10	475	22,700	42,478,913
10 - >5	1,434	33,900	66,368,769
5 - >2.5	6,103	75,200	0
2.5 - >1	19,781	240,000	0
1 - >0.5	39,064	246,000	0
0.5 - >0.25	95,883	350,000	0
0.25 - >0.1	269,883	1,229,000	0
0.1 - 0	34,316,299	0	34,977,809
Subtotals	34,748,633	2,210,000	158,679,135
Total	195,637,768		

[a] Source: Anderson et al., 1980

population dermally exposed to toluene from these sources. The usage of other consumer product formulations containing toluene (see Table 4-20) may cause inhalation/dermal exposure to toluene. An estimate of the number of people exposed to toluene from these products is also unavailable.

According to the estimate of the Department of Health, Education, and Welfare (1977), more than 4.8 million people per year are occupationally exposed to toluene. Toluene ranks fourth among all other agents listed in terms of the number of people exposed to any single agent.

The number of people in the U.S. exposed to toluene through cigarette smoke has been estimated to be 56 million during the year 1978[a]. This figure which considers the exposure to the smokers only, is bound to be an underestimate since it does not include passive smokers.

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[a] This figure is based on the following assumptions: Of the total population of 225 million, 21.4% are under age 13 (Dept. Commer., 1979) and do not smoke. Teenagers in the age group 13 to 17 years constitute 7.6% of the total population (Dept. Commer., 1979). Of the 7.6% of the teenagers, only 11.7% are assumed to be smokers (PHS, 1980). Of the remaining population, 51% are assumed to be females and 49% to be males (Dept. Commer., 1979). The percent of female and male smokers over age 17 are assumed to be 30.4% and 37.4%, respectively (PHS, 1980).

10. ESTIMATE OF HUMAN EXPOSURE

Exposure is the contact between a subject of concern and an agent such as a chemical, biological, or physical entity. The magnitude of the exposure is determined by measuring or estimating the amount of an agent available at the exchange boundaries, that is, lung, gut, and skin, during some specified time. Exposure assessment is the qualitative estimation or quantitative determination of the magnitude, frequency, duration, and route of exposure. Exposure estimates are often combined with environmental and health effects data in performing risk assessments. The exposure of an agent may lead to the intake of some of the agent. Uptake of an absorbed dose is the amount of the intake that is absorbed by the subject.

The assessment of human health risks from exposure to any environmental pollutant requires knowledge of (1) the dosage of the pollutant received by the exposed human population and (2) the effect of the pollutant on human health. Because it is not the purpose of this section to develop a health effects model, no attempt will be made to address such parameters as population characteristics (e.g., age, sex, occupation, racial background), population habits (e.g., food habits, recreational habits, product-use habits), and population groupings (e.g., the aged, pregnant women, children, other high health risk groups). Instead, this section will attempt to derive the human exposure of toluene received from all sources of emissions.

To estimate human exposure, one must consider route of entry, magnitude of exposure, frequency of exposure, and duration of exposure. The general population may be exposed to toluene through the following routes: (1) inhalation of air, (2) ingestion of water and foods, and (3) exposure through skin. The next step combines the estimation of environmental concentrations with a description of the exposed population to yield exposure profiles and exposure pathway analysis.

Certain segments of population may be exposed to toluene through occupational exposure and cigarette smoking. Because exposure of this segment falls under a special category, these scenarios will be discussed separately. This section does not include toluene exposure from the use of consumer products. As has been mentioned in Subsection 10.5., some consumer products contain high percentages of toluene.

Undoubtedly, the use of these consumer products leads to various degrees of toluene exposure in the general population; however, no data are available to derive estimates of toluene exposure from consumer products. Also, the conversion factors for expressing toluene concentrations in air at 25°C are: 1 ppm ≑ 3.77 mg/m^3 and in water, 1 ppm ≑ 1 mg/ℓ.

10.1. EXPOSURE VIA INHALATION

Toluene exposure via inhalation can be estimated in two ways. First, the exposure can be estimated from the total nationwide toluene emission data by the use of mathematical models simulated to reflect the actual environmental setting. Second, the exposure can be estimated from actual monitoring data. Estimating exposure on the basis of monitoring data is often a preferred method, because these data directly provide the environmental distribution of toluene; however, this method has limitations. Although the monitoring data available for toluene are more abundant than those available for many other organic chemicals, they may not be statistically representative of all the population exposed to toluene. The monitoring data may not provide information on the extent of concentration variation due to chemical reactivity (e.g., photoreaction, oxidation in the atmosphere, etc.). These data also do not yield relationships between materials balance of the emitted toluene and the environmental concentration distribution in an area. Therefore, the approach toward exposure estimation in this section has used both the available ambient monitoring data and the theoretical dispersion modeling of toluene emission data.

10.1.1. Theoretical Modeling. The estimation of inhalation exposure to toluene among different segments of the general population involves the following computational tasks: (1) estimation of annual average toluene concentration in the air at different distances from the emission sources and (2) estimation of the population distribution around each source of emission (available through the U.S. Census Bureau). The latter computation has already been discussed in Chapter 9.

The performance of the first task requires the following data: (1) emission inventories of toluene, which are already available (Subsections 4.4.1. through 4.4.4.), (2) atmospheric reactivity of toluene, (3) meteorological data, which are available through the U.S. or local weather bureau, and (4) a dispersion equation to estimate concentration distribution of toluene.

Toluene concentration downwind from a source can be estimated using the following dispersion equation (Turner, 1969):

$$C(X,0,0) = \frac{Q}{\sigma_y \sigma_z U_w} \exp \frac{-h^2}{2\sigma_z^2}$$

where

C(X,0,0) = concentration of toluene at various x coordinates and at zero y and z coordinates (mg/m^3)

Q = emission rate (mg/s)

σ_y = horizontal dispersion coefficient of the plume concentration distribution

σ_z = vertical dispersion coefficient of the plume concentration distribution

U = the mean wind speed (m/s) (w = the heat of the source)

h = the effective stack height; i.e., the sum of the stack height and plume rise (m)

Assuming U = 5 m/s; Q = 200×10^6 kg/year = 6.34×10^3 mg/s; plume height = 10 m and 20 m; and the values of σ_y and σ_z from the following equation (Anderson et al., 1980):

$$\sigma_z \text{ (m)} = 0.06x(1 + 0.0015x)^{-1/2}$$
$$\sigma_y \text{ (m)} = 0.08x(1 + 0.0001x)^{-1/2}$$

one can calculate the concentration of toluene at different distances from the source, as given in Table 10-1.

TABLE 10-1

Concentration of Toluene (mg/m^3) at Different Distances (m) From A Source Emitting 200 Million kg/Year Toluene[a]

Plume Height (m)	100	500	1,000	1,500	5,000	10,000
10	1.36	0.45	0.15	0.12	0.02	0.01
20	0.003	0.31	0.13	0.10	0.02	0.01

[a] Source: Slimak, 1980

The calculations of the values in Table 10-1 for toluene distribution from a stationary source do not consider the chemical reactivity of toluene in the atmosphere and the effect of plume temperature on the concentration distribution of toluene. A more detailed calculation that incorporates these two variables, as well as building wake effect (enhanced dispersion due to buildings), has been made for the estimation of spatial concentration of toluene from the major stationary sources of toluene emission (Anderson et al., 1980).

The dispersion equation developed by Anderson et al. (1980) was used to compute annual average concentration pattern of toluene from each point source. A computer program was used to evaluate these concentration patterns from the given meteorological and emission data. Because there are numerous sources of emission, the sources were divided into three types, each of which is defined below.

Specific Point Sources: These sources were treated using parameters appropriate to each source. Included are emissions from production sources and from chemical intermediate users.

General Point Sources: For these sources, a prototype analysis was done and the results were multiplied by the estimated number of sources. These sources included emissions from gasoline marketing, from the coke-oven industry, and from isolated and non-isolated toluene producers (not included in the previous categories).

Area Sources: Such sources were treated as emission per unit area over identified areas. These sources included mobile emission, emission from solvent use, and emissions from miscellaneous sources.

The three equations used to calculate the spatial concentration distribution of toluene from all sources are given in considerable detail in Anderson et al. (1980); interested readers are referred to that document. The final results of the calculations of Anderson et al. (1980) led to the estimate of spatial concentration range of toluene around different sources of emissions. These values are given in Table 10-2.

TABLE 10-2

Population Distribution and Inhalation Exposure Levels of Toluene From Different Sources[a]

Concentration Level ($\mu g/m^3$)	Number of People Exposed From		
	Specific Point Sources	Prototype Point Sources	Area Sources
>100	0	159	58,347
100 to >50	0	2,841	446,793
50 to >25	34	10,200	12,348,504
25 to >10	475	22,700	42,478,913
10 to >5	1,434	33,900	68,368,769
5 to >2.5	6,103	75,200	0
2.5 to >1	19,781	240,000	0
1 to >0.5	39,064	246,000	0
0.5 to >0.25	95,560	350,000	0
0.25 to >0.1	269,883	1,229,000	0
0.1 to 0	34,316,299	0	34,977,809
Subtotals	34,748,633	2,210,000	158,679,135
Total		195,637,768	

[a]Source: Anderson et al., 1980

Anderson et al. (1980) listed the following factors that could cause uncertainties in their calculated exposure levels given in Table 10-2:

Emission Estimates Errors: Some of these are (1) error in the estimates of production and use of toluene, (2) the assumption that all plants operate at the same capacity, (3) omission of certain emission sources, and (4) error in derivation of emission factors and, in certain cases, the use of a uniform emission factor, which implies that all these plants have similar emission controls. It is difficult to project whether the emission estimates used by Anderson et al. (1980) will lead to higher or lower exposure estimates. This can be done, however, by comparing these estimates with the experimentally determined concentration patterns obtained from sources that are reasonably isolated from other sources.

Concentration Pattern Errors: The concentration patterns used in the exposure computations were obtained through atmospheric dispersion modeling. Any deviations in these estimates from the true pattern (difference in theoretical and experimental values) directly affect the exposure results. Many assumptions were used in calculating the concentration distribution. The exposure errors will be more severe in the case of prototype point sources where a prototype model was used for calculating exposure from all other similar sources. The same can be said about the exposure estimates from area sources based on a box model method that incorporated a number of uncertainties.

Interpolation Errors: The interpolation of population and concentration patterns used to develop patterns of exposure can introduce errors.

With the available information, it is impossible to quantify any of the errors described above. The theoretical model may provide qualitative insights in certain instances to predict whether the exposure estimate is either too high or too low compared to the actual values.

10.1.2. Inhalation Exposure Based on Monitoring Data. Exposure of the general population to toluene by inhalation can occur under a wide range of exposure scenarios. Because it may be considered impractical to measure toluene concentration from all possible exposure scenarios, an attempt has been made to develop a few of the most prevalent ones.

The four largest sources of toluene emission, in descending order, are automobile use (exhaust emission, engine evaporative loss, gasoline marketing evaporative loss), industry sites using toluene as a solvent, coke oven sites, and toluene production sites (Subsection 4.4.4.). In place of dispersion modeling, one can use the monitoring data from each of the four sites to evaluate the four different exposure scenarios. The difficulty with this approach is that the available monitoring data were often developed for sites with various degrees of intermixing between these exposure scenarios. Therefore, inhalation exposure has been classified under three scenarios: the urban areas, areas containing the user sites, and rural or remote areas. In this manner, the exposure estimates developed may be representative of a broad range of the possible exposure scenarios. It should be remembered that the urban areas may contain sites with high automobile use, production and other manufacturing sites, and coke-oven sites.

Human exposure to toluene through inhalation of urban air is shown in Table 10-3. The concentration of toluene in urban areas in the United States in recent years ranged from 0.1 $\mu g/m^3$ to 204 $\mu g/m^3$ (Table 7-1). The intake estimate is based on a breathing rate of 1.2 m^3/hour for an adult during waking hours and 0.4 m^3/hour during sleeping hours (Slimak, 1980). It is also assumed that the sleeping period for an adult is 8 hours/day. This results in an inspired volume of $(1.2 \times 16 \times 7 + 0.4 \times 8 \times 7) = 156.8$ m^3/week.

Near user sites, the range of toluene concentration has been assumed to be 5.5 to 600 $\mu g/m^3$. This range corresponds to the measured value of Sexton and Westberg (1980) near an automotive painting plant (Subsection 7.1.1.) (solvent use constitutes about 99% of total usage). The concentration of toluene at a distance 18 km from the plant measured 55.5 $\mu g/m^3$, a value 10 times higher than the background concentration (Sexton and Westberg, 1980). Therefore, even workers who commute more than 18 km from the plant are susceptible to inhaling toluene in the concentration range of 5.5 to 600 $\mu g/m^3$ for the entire 168 hours in a week. The toluene concentrations near manufacturing sites range from 0.1 to 147 $\mu g/m^3$. The estimated toluene exposure range from the manufacturing and user sites shown in Table 10-3 is based on a concentration range of 0.1 to 600 $\mu g/m^3$.

In rural and remote areas, the concentration of toluene has been reported to be in the range of a trace to 3.8 $\mu g/m^3$ (Table 7-1). These concentrations were determined in 1971; the current level may be lower than this range, as indicated

TABLE 10-3

Toluene Exposure Under Different Exposure Scenarios

Scenario	Observed Range of Concentration	Frequency of Exposure	Total Volume Exposed or Amount Consumed	Inhalation or Ingestion Rate (mg/wk)
General Population				
Inhalation				
Urban areas	0.1 to 204 μg/m^3	168 h/wk	156.8 m^3	0.02 to 32
Rural and remote areas	trace to 3.8 μg/m^3	168 h/wk	156.8 m^3	trace 0.6
Areas near manufacturing and user sites	0.1 to 600 μg/m^3	168 h/wk	156.8 m^3	0.02 to 94
Ingestion				
Drinking water	0 to 19 μg/ℓ	2 ℓ/d	14 ℓ	0 to 0.3
Food (fish only)	0 to 1 mg/kg	6.5 g/d	45.5 g	0 to 0.45
Occupational Group				
Inhalation	377,000 μg/m^3[c]	40 h/d	48 m^3	18,100
Dermal	0 to 170 μg/ℓ[b]	0 to 30 min/wk	5.9 ℓ	0 to 1.0
Cigarette Smokers				
Inhalation	0.1 mg/cigarette	20 cigarettes/d	140 cigarettes	14

[a] This value is the OSHA recommended standard and represents the worst-case estimate. In some industries, the exposure level rarely exceeds 10 ppm.

[b] This value represents exposure to blood due to dermal contact and represent absorbed levels.

[c] This value is the OSHA recommended standard.

h = hour; wk = week; d = day; min = minute by the toluene concentration reported at Grand Canyon in 1979. The estimated toluene exposure in rural and remote areas is shown in Table 10-3.

It should be remembered that Table 10-3 shows the amount of toluene inspired per week by humans around certain exposure scenarios and not the amount absorbed. Only a certain fraction of the toluene inhaled is absorbed by human organs. Also, part of the absorbed toluene is rapidly excreted from the body.

10.2. INGESTION EXPOSURE BASED ON MONITORING DATA

No theoretical modeling method is available for estimating toluene exposure from various ingestion sources. Therefore, the exposure estimate from this source has been attempted by using the limited monitoring data that are available.

10.2.1. Exposure from Drinking Water. The concentrations of toluene in drinking water range from 0 to 19 µg/ℓ (Subsection 7.1.2.5.). The concentration of toluene measured in well waters in New York State was below 10 µg/ℓ (Subsection 7.1.2.4.). Therefore, a concentration range of 0 to 19 µg/ℓ has been used for exposure assessment shown in Table 10-3. A consumption rate of 2 ℓ/day has also been assumed for exposure asessment.

10.2.2. Exposure from Edible Aquatic Organisms. The concentration range of toluene in edible aquatic organisms has been assumed to be 0 to 1 mg/kg, based on the level of toluene found in unspecified fish tissues (Subsection 7.1.4.). On the basis of these data and the assumption that the per capita consumption of aquatic organisms in the United States is approximately 6.5 g/day (Stephan, 1980), the exposure range of toluene from food is shown in Table 10-3.

10.3. OCCUPATIONAL EXPOSURE

Occupational exposure to toluene can primarily take place from inhalation of air containing toluene and from skin contact with toluene or other solvent mixtures containing toluene. The concentration of toluene in the air of the work place has been assumed to be 377,000 µg/m^3. This value corresponds to the NIOSH (National Institute for Occupational Safety and Health) recommended workroom air standard of 100 ppm toluene vapor as a time-weighted average (TWA) exposure for an 8-hour work day (NIOSH, 1973). This value is reasonably close to the actual occupational exposure levels discussed in Section 7.2. Based on the above assumptions, the inhalation exposure of toluene by occupational groups as shown in Table 10-3 far exceeds that for any other group.

Sato and Nakajima (1978) studied the absorption of toluene through human skin. These investigators immersed one hand of 5 male subjects in pure toluene for 30 minutes and monitored the blood levels of toluene. A peak concentration of 170 µg/ℓ of blood was observed after a 30-minute immersion. This maximum concentration was maintained for 10 to 15 minutes after exposure had ended and decreased thereafter.

Although the standard proposed by NIOSH (1973) requires all workers handling toluene to wear gloves, it is conceivable that short-term exposure of bare skin to toluene takes place under certain circumstances. For assessment of exposure through skin as shown in Table 10-3, a maximum concentration of 170 µg/ℓ in blood and a blood volume of 5.9 ℓ for an adult male have been assumed. It has also been assumed that the skin exposure duration does not exceed 30 minutes/week. It also should be recognized that the value for blood concentration through dermal contact given in Table 10-3 does not represent the total exposure value, as it ignores exposure to other organs.

10.4. CIGARETTE SMOKERS

The concentration of toluene in inhaled cigarette smoke has been determined to be 0.1 mg/cigarette (see Subsection 7.3). In assessing toluene exposure from cigarette smoking, it was assumed that an individual smokes 20 cigarettes per day. On the basis of these assumptions, it can be predicted from Table 10-3 that cigarette smoking may be the second largest source of human exposure to toluene.

10.5. LIMITATIONS OF EXPOSURE ESTIMATE BASED ON MONITORING DATA

As discussed earlier, exposure estimates on the basis of monitoring data have the following limitations:

(1) The limited monitoring data do not provide information for estimating exposure under different exposure scenarios. Even when some data are available, they may be inadequate and even susceptible to error. It is very difficult to assess the errors in the monitoring data.

(2) The monitoring data often do not relate to the source of emissions in terms of material balancing of the amount emitted and the concentration measured.

(3) The population distribution around the monitoring area is rarely provided in these data, although such data may be available independently of monitoring.

(4) The estimate for toluene exposure to the general population from food and drinking water as given in Table 10-3, is very crude. Toluene has been detected in only a small fraction of total drinking water supplies monitored (Subsection 7.1.2.5.). The exposure estimate does not specify either the number of people or the locations where people are exposed to toluene from drinking water. The same can be said with respect to toluene exposure from food.

10.6. COMPARISON BETWEEN EXPOSURE DATA BASED ON THEORETICAL AND EXPERIMENTAL VALUES

If the concentration values ranging from 0 $\mu g/m^3$ to greater than 100 $\mu g/m^3$ (Table 10-2) are combined with the value of 156.8 m^3 for inspired volume of air per week, an inhalation exposure estimate as shown in Table 10-4 can be developed.

TABLE 10-4

Exposed Population and Exposed Amount of Toluene From Dispersion Modelling[a]

Concentration Level ($\mu g/m^3$)	Exposed Concentration mg/week
>100	>15.7
100 to 10	15.7 to 1.6
10 to 1	1.6 to 0.15
1 to 0.1	0.15 to 0.02
0.1 to 0	0.02 to 0

[a] Source: Slimak, 1980

A comparison of inhalation exposure data shown in Table 10-4, which are based on dispersion equations, with inhalation exposure data in Table 10-3, which are derived from monitored concentrations, shows reasonable agreement between the two sets of data. The monitoring data estimate toluene inhalation by the general population in urban areas to be 0.02 to 32 mg/week. The exposure data developed from dispersion equations estimate this value to be in the range of zero to greater than 15.7 mg/week. The cumulative inhalation exposure can be calculated by multiplying the exposed concentrations from Table 10-4 with the appropriate exposed population given in Table 10-2.

10.7. REFERENCES

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11. EFFECTS ON HUMANS

Human exposure to toluene primarily involves inhalation, and consequently the effect of greatest concern is dysfunction of the CNS. As detailed in Chapters 9 and 10, millions of individuals are exposed to toluene via inhalation of air from ambient atmosphere and cigarette smoke (ppb concentrations), and from occupational exposures (ppm concentrations). Toxicity studies of humans have centered, however, on evaluation of individuals exposed to toluene in experimental and occupational settings, and from deliberate inhalation of toluene or toluene-containing substances ("glue sniffing"). It should be noted that occupational exposures and glue sniffing often involve complex mixtures of solvents, and that prior to the 1950s, benzene was a common contaminant of toluene. In evaluating the effects of toluene exposures, the purity of the compound must be considered.

Glue sniffers inhale the vapors from a wide variety of volatile hydrocarbons (usually poorly defined mixtures) contained in products such as glues and thinners for their euphoric or intoxicating effects. The most popular of these products contains toluene, and toluene is the hydrocarbon most frequently implicated as the cause of the adverse effects associated with deliberate inhalation. The practice has been reviewed extensively (Massengale, 1963; Barman et al., 1964; Press and Done, 1967a, 1967b; Gellman, 1968; Wyse, 1973; Linder, 1975; Faillace and Guynn, 1976; Oliver and Watson, 1977; Walter et al., 1977; Watson, 1979). Excessive levels of toluene generally are inhaled over a short time interval, and repeated inhalation of the vapors is associated with the development of tolerance and psychological dependence. The most common methods of inhalation involve (1) placing the solvent in a plastic bag and inhaling the fumes, (2) soaking a rag or handkerchief with the solvent and sniffing the rag, and (3) sniffing the solvent from a container. The concentrations of toluene inhaled under these conditions can approach 30,000 ppm (i.e., saturation concentration at 20°C).

11.1. EFFECTS ON THE NERVOUS SYSTEM

11.1.1. Central Nervous System

11.1.1.1. ACUTE EFFECTS -- Experimental exposures of up to 800 ppm toluene have produced acute dose-related CNS alterations (Von Oettingen et al., 1942a, 1942b; Carpenter et al., 1944). Von Oettingen et al. (1942a, 1942b) provided what generally is acknowledged to be the most complete description of the effects of pure toluene (benzene $\leq$ 0.01%) on the CNS. In single 8-hour exposures, 3 human subjects were subjected to concentrations of toluene in an exposure chamber that ranged from 50 to 800 ppm (Table 11-1). A maximum of two exposures a week were conducted to allow sufficient time in between for recovery; a total of 22 exposures was performed over an 8-week period. Seven of the 22 exposures were to pure air, and exposures to particular levels of toluene were replicated only 1 to 4 times. The effects observed are summarized in Table 11-1. Subjective complaints of fatigue, muscular weakness, confusion, impaired coordination, and enlarged pupils and accommodation disturbances were reported at levels of 200 ppm. These effects increased in severity with increases in toluene concentration, until at 800 ppm the subjects experienced severe fatigue, pronounced nausea, mental confusion, considerable incoordination and staggering gait, strongly impaired pupillary light reflex, and after-effects (muscular fatigue, nervousness, and insomnia) that lasted for several days.

Carpenter and coworkers (1944) exposed 2 male subjects to known concentrations of toluene (purity not stated) for periods of 7 to 8 hours and noted slight exhilaration at 200 ppm, and lassitude, nausea, and hilarity at 400 ppm. Lassitude, hilarity, verbosity, and boisterousness occurred at 600 ppm (anorexia and listlessness were reported as after-effects.), and transitory headaches, extreme lassitude, scotomata (areas of depressed vision), verbosity, slight nausea, and "inebriation" were found at 800 ppm. Marked unsteadiness was also observed in the subjects during exposure to 800 ppm toluene. Steadiness was determined by a test that involved holding at arms' length a wire in a hole for 3 minutes; the percentage of time the wire was actually in contact with the side of the hole was determined, and compared with the normal value from each test session.

Short-term experimental exposures to toluene have also elicited increases in reaction time and reductions in perceptual speed (Ogata et al., 1970; Gamberale and Hultengren, 1972). Ogata and coworkers (1970) reported that 23 Japanese subjects given single exposures to 200 ppm toluene showed a prolonged eye-to-hand reaction time, but no effect on flicker fusion frequency. Exposures were for 3 hours, or 3 hours and a 1 hour break period followed by 4 additional hours of exposure. No changes in either reaction time or flicker value were observed at 100 ppm. It should be noted, however, that no other information regarding the design of these experiments was presented.

TABLE 11-1

Effects of Controlled 8 Hour Exposures to Pure Toluene on Three Human Subjects[a,b]

Concentration	No. of Exposures	Effects
0 ppm (control)	7	No complaints or objective symptoms, except occasional moderate tiredness toward the end of each exposure, which was attributed to lack of physical exercise, unfavorable illumination, and monotonous noise from fans.
50 ppm	2	Drowsiness with a very mild headache in 1 subject. No aftereffects.
100 ppm	4	Moderate fatigue and sleepiness (3), and a slight headache on one occasion (1).
200 ppm	3	Fatigue (3), muscular weakness (2), confusion (2), impaired coordination (2), paresthesia of the skin (2), repeated headache (1), and nausea (1) at the end of the exposure. In several instances, the pupils were dilated, pupillary light reflex was impaired, and the fundus of the eye was engorged. Aftereffects included fatigue, general confusion, moderate insomnia, and restless sleep in all 3 subjects.
300 ppm	2	Severe fatigue (3), headache (2), muscular weakness and incoordination (1), and slight pallor of the eyeground (2). Aftereffects included fatigue (3) and insomnia (1).
400 ppm	2	Fatigue and mental confusion (3), headache, paresthesia of the skin, muscular weakness, dilated pupils, and pale eyeground (2). Aftereffects were fatigue (3), skin paresthesia (1), headache (1), and insomnia (2).
600 pp[illegible]	1	Extreme fatigue, mental confusion, exhilaration, nausea, headache and dizziness (3), and severe headache (2) after 3 hours of exposure. After 8 hours' exposure, the effects included considerable incoordination and staggering gait (3), and several instances of dilated pupils, impaired pupillary light reflex and pale optic discs; aftereffects included fatigue and weakness, nausea, nervousness and some confusion (3), severe headache (2), and insomnia (2). Fatigue and nervousness persisted on the following day.

TABLE 11-1 (cont.)

Concentration	No. of Exposures	Effects
800 ppm	1	Rapid onset of severe fatigue and, after 3 hours, pronounced nausea, confusion, lack of self-control, and considerable incoordination and staggering gait in all 3 subjects. Also, pupillary light reflex was strongly impaired (1) and optic discs were pale (2). All 3 subjects showed considerable aftereffects, lasting at least several days, which included severe nervousness, muscular fatigue, and insomnia.

[a] Source: Von Oettingen et al., 1942a, 1942b

[b] Exposures were twice weekly for 8 weeks. The number of subjects affected is noted in parentheses.

In a more extensive study, Gamberale and Hultengren (1972) exposed 12 male subjects to 100, 300, 500, or 700 ppm toluene (via breathing valve and mouthpiece) during successive 20-minute exposure periods, and measured their performance on four tests of perceptual speed and reaction time at each level of exposure (Table 11-2). The tests were always made in the same sequence (i.e., Identical Numbers, Spokes, Simple Reaction Time, Choice Reaction Time) during the final 15 minutes of each exposure period. Toluene concentrations were increased from 100 to 300 ppm and from 500 to 700 ppm without interruption, but the increase from 300 to 500 ppm was made following a 5-minute interval without exposure. Menthol crystals contained in the mouthpiece tubing camouflaged the taste and smell of the toluene. The 12 subjects were divided into two groups of equal size: subjects in one group were studied individually, first under experimental conditions with exposure and then under control (atmospheric air containing menthol) conditions 7 days later, while subjects in the other group were studied under similar conditions but in the reverse order. The camouflage of the inspiratory air with menthol made it impossible for 11 of the 12 subjects to distinguish between exposure to toluene and exposure to pure air.

Results of the Gamberale and Hultengren (1972) study showed that both reaction time and perceptual speed were impaired during exposure to toluene as compared to exposure to pure air (Table 11-2). With respect to reaction time, a significant effect was noted upon exposure to 300 ppm toluene in one test (Simple Reaction Time), and a performance decrement, which reportedly approached statistical significance at the 0.05 level, was noted for the other test (Choice Reaction Time). Subject reaction time was further impaired at higher levels of exposure (500 and 700 ppm toluene), but no impairment in either reaction time test was noted for exposure to 100 ppm. (The 100 ppm reaction time no-effect level is consistent with the aforementioned results of Ogata et al., 1970.) No statistically significant impairment in subject perceptual speed was observed until the concentration of toluene in the inspiratory air was 700 ppm. Because perceptual speed was unaffected at concentrations below 700 ppm, the authors suggested that the simpler CNS functions may be affected at lower levels of toluene exposure than the more complex functions.

Winneke et al. (1976) noted, in an abstract published in the Proceedings of the 2nd International Industrial and Environmental Neurology Congress (Prague, Czechoslovakia), that experimental exposure to 98 ppm toluene for 3 hours did not affect psychophysiological performance in 20 subjects. The parameters

TABLE 11-2

Effect of Toluene Exposure on the Performance of Perceptual Speed and Reaction Time Tests[a,b]

Performance Test	Concentration (ppm)	Mean Test Scores		t-Value
		Experimental Conditions	Control (Air) Conditions	
Identical Numbers[c] (minutes)	100	5.62	5.53	+0.50
	300	5.25	5.29	-0.39
	500	5.13	5.04	+1.34
	700	5.19	4.80	+2.65*
Spokes[d] (seconds)	100	50.5	50.8	-0.08
	300	46.7	43.7	+1.18
	500	43.6	40.2	+1.28
	700	45.4	36.9	+2.51*
Reaction Time - Simple[e] (meters/second)	100	228	230	-0.31
	300	236	222	+2.35*
	500	246	219	+3.88**
	700	253	214	+4.81**
Reaction Time - Choice[f] (meters/second)	100	425	422	+0.34
	300	429	416	+1.99
	500	432	400	+2.91*
	700	442	408	+3.59**

[a] Source: Gamberale and Hultengren, 1972

[b] 12 male subjects were exposed to toluene concentrations of 100, 300, 500, and 700 ppm during four successive 20-minute periods. The tests were performed at each concentration sequentially in the order listed. The number of times each test sequence was repeated was not stated.

[c] Perceptual speed: Identical Numbers. Subjects were instructed to underline the 3-digit number, from a total of 60 columns, that was identical to the number at the head of each column. Performance was measured as the time taken to complete the test.

[d] Perceptual speed: Spokes. Subjects were instructed to connect circles located at random on four pages and numbered from 1 to 20 in the correct numerical order using a pen. Performance was measured as the mean time taken for the four assignments.

[e] Simple Reaction Time. Subjects were instructed to respond to a signal from a lamp by pressing a pushbutton. Stimuli were administered at intervals of approximately 10 seconds, an acoustic warning signal was given 3 seconds prior to onset of stimuli, and 30 stimuli were given in each trial. Performance was measured as the mean reaction time for the last 20 stimuli administered.

[f] Choice Reaction Time: Stimulus/reply test as above, but there were three pushbuttons equipped with matching stimulus lamps. Stimulus administration followed a random sequence with the number of light signals evenly distributed among the lamps, but the trial and performance measurements were otherwise the same as for simple reaction time.

Degrees of freedom = 11; *P < 0.05; **P < 0.01; ***P < 0.001

evaluated in this study included performance in a bisensory (auditory and visual) vigilance task, psychomotor performance, critical flicker frequency, and auditory evoked potentials. The available abstract did not provide any additional information on the experimental design, the nature of the psychophysiological tests, or the results of this study.

Gusev (1965) examined the effects of acute low-level toluene exposure on the electroencephalographic (EEG) activity of four human subjects who were trained to develop synchronous and well-marked alpha rhythms when stimulated by light. Toluene exposures of approximately 0.27 ppm (1 mg/m^3) for 6 minutes apparently caused statistically distinct changes in EEG activity from the left temporal-occipital region in all subjects; these changes persisted through a 6-minute recovery period. It should be noted that the 0.27 ppm concentration is slightly lower than the odor threshold determined for toluene in the same experiment 0.40 ppm; see subsection 11.7.2.). Toluene concentrations of 0.16 ppm (0.6 mg/m^3) caused no variations in the electric potentials of the EEGs. Exposure sessions consisted of 10 separate observation periods in which inhalation of toluene (5 periods) alternated with inhalation of pure air (5 periods). A single period consisted of 18 one-minute cycles. Every cycle included the sequential presentation of a sound stimulus (10 seconds), a wait for the light stimulus (7 seconds), the presentation of the light stimulus (18 seconds), and an interval of active physical exercise (25 seconds) for recovery of normal EEG rhythm. Of the 18 minutes allotted for EEG recording in each period, 3 minutes were used for training, the next 3 minutes for background observations, the following 6 minutes for the toluene exposure, and the final 6 minutes for recovery. Although the reported effects on EEG activity may represent a subtle indication of perception, there is no apparent toxicological significance to the finding. It should further be noted that western studies have not reported any effect of toluene on the CNS at such low levels of exposure, and that the purity of the toluene used was not stated.

Narcosis is the primary result of acute toluene exposure at high concentrations. A number of accounts of workers who were rendered unconscious by toluene vapor have been published in the medical literature (Lurie, 1949; Browning, 1965; Longley et al., 1967; Reisin et al., 1975). Most of these cases have involved the entry of workmen into confined areas with poor ventilation and subsequent exposure to high levels of toluene during maintenance operations. Longley et al. (1967) described two episodes of acute toluene intoxication involving 26 men who were exposed in the holds of cargo ships. Toluene concentrations were estimated to have ranged from 10,000 ppm at waist level to 30,000 ppm at floor level, but it was emphasized that this estimate was purely conjectural. Effects at these concentrations ranged from exhilaration, lightheadedness, and clumsiness and dizziness to collapse and unconsciousness. No deaths occurred and recovery was quite rapid, with no after-effects following removal from the contaminated atmosphere. The durations of the exposures were not indicated, but loss of consciousness occurred within minutes.

Episodes of toluene abuse are characterized by the progressive development of CNS symptoms. Toluene sniffers experience an initial excitatory stage that is typically characterized by drunkenness, dizziness, euphoria, delusions, nausea and vomiting, and, less commonly, visual and auditory hallucinations (Press and Done, 1967a, 1967b; Wyse, 1973; Lewis and Patterson, 1974; Hayden et al., 1977; Oliver and Watson, 1977; Barnes, 1979). As duration of exposure increases, symptoms indicative of CNS depression become evident: confusion and disorientation, headache, blurred vision and reduced speech, drowsiness, muscular incoordination, ataxia, depressed reflexes, and nystagmus. In extreme cases, loss of consciousness, possibly with convulsions (Helliwell and Murphy, 1979), occurs. The duration and severity of these effects vary greatly, depending upon the intensity of exposure; the duration may range from 15 minutes to a few hours (Press and Done, 1967b). Also, all of the symptoms described have not been exhibited in any single sniffer, nor in any single episode of sniffing.

Winek et al. (1968) published partial results of an autopsy on an adolescent who had died as a result of sniffing model airplane glue containing toluene. At autopsy, the cut surfaces of the lungs of this individual were found to be extremely frothy and congested, with diminished amounts of crepitation throughout the lung tissue. Other gross observations that were noted included some petechial hemorrhages in the larynx and upper trachea, firmness and congestion in the spleen, and a dark, red-brown color and congestion in the liver. No hemorrhages, obstructions, or ulcerations were seen anywhere in the gastrointestinal tract, and all other organs were unremarkable. The results of toxicological analyses of various body tissues for toluene are presented in Section 13.2. Congestion in various organs, swelling of the brain, subseromucous petechiae, and pulmonary edema were associated with 19 other cases of acute death from thinner intoxication (Chiba, 1969); the English abstract of this Japanese study indicated that toluene was the major component of the inhaled thinner. Nomiyama and Nomiyama (1978) described an instance in which 4 adolescents were found dead after sniffing 99% pure toluene in a car, but post-mortem results other than levels of toluene (blood and alveolar air) and hippuric acid (urine) were not presented. Sudden death due to solvent sniffing has been reported in at least 122 cases (Bass, 1970; Alha et al., 1973). The sudden deaths have been attributed, however, to severe cardiac arrhythmia, and are discussed in Subsection 11.5. (Effects on the Heart).

11.1.1.2. SUBCHRONIC AND CHRONIC EFFECTS -- Wilson (1943) described the effects of exposure to commercial toluene vapor on 100 workers (out of a total of 1000 workers) who showed symptoms severe enough to seek examination at a hospital. The workers were exposed daily to toluene concentrations ranging from 50 to 1500 ppm for periods of 1 to 3 weeks, but the composition of the commercial formulation and the type of industry were not described. Also, it is unclear whether the remaining 900 workers evidenced any symptoms of toluene exposure. The concentration of toluene was determined shortly after any exposed person appeared at the hospital with symptoms, and the patients were classified into groups by degree of exposure. The following effects were reported:

50 to 200 ppm (approximately 60% of the patients) - headache, lassitude, and loss of appetite. These symptoms were so mild that they were considered to be due primarily to psychogenic and other factors rather than to toluene fumes.

200 to 500 ppm (approximately 30% of the patients) - headache, nausea, bad taste in the mouth, anorexia, lassitude, slight but definite impairment of coordination and reaction time, and momentary loss of memory.

500 to 1500 ppm (approximately 10% of the patients) - nausea, headache, dizziness, anorexia, palpitation, and extreme weakness. Loss of coordination was pronounced and reaction time was definitely impaired.

Characteristic CNS alterations have also been described in foreign reports of workers exposed for longer durations to moderate levels of toluene. Parmeggiani and Sassi (1954) found signs of "nervous hyperexcitability" in 6 out of 11 paint and pharmaceutical industry workers who were exposed to 200-800 ppm toluene vapor for "many" years. Capellini and Alessio (1971) noted symptoms of stupor, nervousness, and insomnia in one worker who was employed for "diverse" years in preparing a toluene-containing mixture for use in the manufacture of V-belts. The mean atmospheric concentration of toluene in the mixing department was 250 ppm, with extremes of 210 ppm and 300 ppm. No CNS effects were observed, however, in 17 other workers who were exposed to 125 ppm toluene (range, 80 to 160 ppm) while engaged in the manufacture of the belts.

In a more extensive study, Suhr (1975) found no evidence of adverse neurological effects in a group of 100 rotogravure printers with at least 10 years of exposure to 200 to 400 ppm pure toluene (<0.3% benzene). Subjective complaints indicative of CNS toxicity (headache, giddiness, nervousness, irritability, sleeplessness, bodily fatigue and incoordination), abnormal reflex reactions, and abnormal Sphallograph test results were not found to occur significantly more often in the printers than in an unexposed control group of equal size. The Sphallograph is an instrument that is used to detect slight disturbances of muscular coordination by sensing variations in the balance of two metal plates; a test person stands on the plates, and balance disturbances are detected by strain gauges.

The Suhr (1975) conclusion that chronic occupational exposure to 200 to 400 ppm toluene did not cause adverse neurological effects in the rotogravure workers is equivocal for several reasons. First, the nature of the control group used in this study is not defined, other than that they "were from the same firm and not exposed to toluene." Additionally, the worker and control groups were only roughly matched by groups for age distribution, years of exposure, and nature of workshift (i.e., 2 or 3 shift work). Second, the venous blood levels measured in the printing room workers at the end of their shifts indicate exposure to toluene levels of at least 300 ppm and possibly as high as 600 ppm. These levels are consistent with the reported air concentration measurements, which were made with a "measuring cell" device. It is unclear, however, when workers were examined for reflex reactions and Sphallograph measurements. If it was after or before the workshifts (as the data for the 33 Sphallograph groups would indicate), then blood levels of toluene may have declined significantly. Astrand et al. (1972) have shown major drops in levels within minutes after the removal of human subjects from exposure. Third, the Sphallograph appears to be a very infrequently used device in the United States; several behavioral toxicologists who were contacted by Syracuse Research Corporation (SRC) indicated that they have never heard of the instrument, and the device does not appear to have been described in standard texts. Suhr (1975) also cites the work of Pohl and

Schmilde (1973), who tested the effects of "extreme" concentrations of 11 frequently used organic solvents in humans with the Sphallograph and found only minimal effects. This would argue that the Sphallograph is not a sensitive test for determining CNS effects of solvents. Last, until more is known concerning the exposures of the control group, the significance of the reportedly negative results of the subjective symptom survey is questionable.

Chronic occupational exposure to toluene has also been associated with behavioral changes. Munchinger (1963) diagnosed an "organic psychosyndrome" in 21% of a group of printers exposed on the average to 300 ppm toluene for 18 years (mean age, 42 years), and in 40% of a group of printers' helpers exposed to 430 ppm for 12 years (mean age, 44 years). A total of 110 workers were examined, but the number of printers and printers' helpers was not stated and testing on control subjects was not performed. The syndrome was characterized by subjective memory, thinking, and activity disturbances. Results of Rorschach testing were consistent with the psychosyndrome diagnosis in 83% of the cases. In combination, Rorschach Test and Knoepfel's 13-Error Test results agreed with the diagnosis in 95% of the cases.

More recently, several groups of investigators have shown that long-term exposure to combinations of toluene and other common organic solvents caused impairments in visual intelligence and psychomotor performance of workers. In 1973, Lindstroem compared the psychological test performances of a group of 168 male workers who had been exposed to hydrocarbon solvents for 0.1 to 30 years (mean, 6 years) to those of an unexposed control group (N = 50). Twenty-six of the workers had been exposed primarily to toluene and 25 others to a combination of toluene and xylene; the remaining workers (numbers in parentheses) were exposed primarily to trichloroethylene (44), tetrachloroethylene (8), "thinners" (44), and miscellaneous solvents (21). Exposure concentrations were not reported. Results showed that the solvent-exposed workers were inferior in performance to the controls in sensorimotor speed performance, psychomotor performance, and visual accuracy as determined by standardized test procedures (e.g., Bourdon-Wiersma vigilance test, Santa Ana dexterity test, Mira psychomotor test). The performance of the workers on the Rorschach personality test was comparable to that of the control group.

Hanninen et al. (1976) compared the behavioral responses of a group of 100 car painters with those of 101 age-matched nonexposed subjects. The painters (mean age 35 ± 11 years) were exposed to different organic solvents for 1 to

40 years (mean, 14.8 ± 8.5 years), but, as detailed in Table 11-3, toluene was present in the greatest amount (30.6 ppm).

TABLE 11-3

Mean Concentrations of Organic Solvents in the Breathing Zone of 40 Car Painters[a,b]

Solvent	Mean Concentration (ppm)
Toluene	30.6
Xylene	5.8
Butyl Acetate	6.8
White Spirit	4.9
Methyl Isobutyl Ketone	1.7
Isopropanol	2.9
Ethyl Acetate	2.6
Acetone	3.1
Ethanol	2.9

[a] Source: Hanninen et al., 1976

[b] Sampling Period = 1 hour; Number of Car Repair Garages = 6; Number of Samples = 54.

A battery of tests included 1 test for verbal intelligence, 3 visual tests, 5 memory or learning tasks, 4 tests of psychomotor performances, and the Rorschach test for measuring personality changes (Tables 11-4 and 11-5). Results of this study showed significant differences between the exposed and reference group in almost all intellectual performances and memory tasks. Impairments in visual and verbal intelligence and in memory, as well as a reduction of emotional reactivity as indicated by the Rorschach test, were the predominant effects of solvent exposure (Tables 11-4 and 11-5). Differences in psychomotor performances between the exposed and control subjects were less consistent; impairments were seen only in some of the Santa Ana dexterity and finger tapping test scores, and

TABLE 11-4

Performance Tests: Means, Standard Deviations, and Significances Between the Group Means (Age-Matched) Groups[a]

Test	Means and Standard Deviations		Significance of Differences (t-test)
	Exposed (N = 100)	Nonexposed (N = 150)	
WAIS[a] Similarities test[c]	19.4 ± 3.1	2.9 ± 2.1	***
WAIS Picture Completion[d]	14.9 ± 2.9	16.2 ± 2.3	***
WAIS Block Design[e]	34.6 ± 7.0	39.6 ± 5.6	***
Figure Identification[f]	32.0 ± 9.0	36.7 ± 9.8	***
WAIS and WMS[g] Digit Span[h]	10.6 ± 1.6	11.5 ± 1.8	***
WMS Logical Memory[i]	11.7 ± 3.7	13.9 ± 3.1	***
WMS Associate Learning[j]	15.3 ± 3.6	17.1 ± 2.6	***
Benton Test for Visual Reproduction	21.1 ± 3.1	22.6 ± 2.3	***
Benton Test for Visual Retention	8.2 ± 1.5	8.7 ± 1.3	*
SADT - right hand[k]	44.7 ± 5.7	47.5 ± 5.8	**
SADT - left hand[k]	42.3 ± 5.4	43.6 ± 5.1	
SADT - coordination with both hands[k]	29.0 ± 5.4	31.5 ± 5.7	**
Finger Tapping - right hand[l]	202.5 ± 29.2	209.6 ± 23.8	
Finger Tapping - left hand[l]	186.7 ± 28.5	196.4 ± 22.4	*
Reaction Time (Simple) - right hand	12.4 ± 2.9	11.9 ± 1.4	
Reaction Time (Simple) - left hand	12.1 ± 3.0	11.7 ± 1.4	
Reaction Time (Choice)	9.1 ± 1.8	9.1 ± 1.2	
Mira Test[m]	18.8 ± 3.8	20.3 ± 4.6	**[n]
Mira Test[m]	2.2 ± 1.0	2.0 ± 0.8	*

[a] Source: Hanninen et al., 1976

[b] Wechsler Adult Intelligence Scale.

[c] Measures verbal intelligence and abstraction.

[d] Measures visual intelligence and observation.

[e] Measures visual intelligence and abstraction.

[f] Measures speed of perception and memory for visual details.

[g] Wechsler Memory Scale.

[h] Measures memory for digits.

[i] Measures verbal memory.

[j] Measures verbal memory and learning.

[k] Santa Ana Dexterity Test; measures psychomotor speed.

[l] Measures motor speed.

[m] Test for psychomotor behavior and psychomotor ability; two variables tested.

[n] Paired t-test.

$*P < 0.05$; $**P < 0.01$; $***P < 0.001$

TABLE 11-5

Rorschach Personality Test Variables: Means, Standard Deviations, and Significances Between the Group Means (Age-Matched Groups)[a]

Variable	Means and Standard Deviations		Significance of Differences (t-test)
	Exposed (N = 100)	Nonexposed (N = 101)	
Number of responses	13.6 ± 6.4	13.8 ± 4.5	
Number of rejections	0.7 ± 1.1	0.4 ± 1.0	**[b]
Average latency time of responses	16.4 ± 8.5	16.5 ± 8.1	
Adaptability	11.6 ± 3.1	12.1 ± 3.1	
Emotionality	8.8 ± 3.3	10.4 ± 3.2	***
Spontaneity	11.8 ± 2.4	11.9 ± 2.6	
Rational self-control	8.6 ± 2.8	7.3 ± 2.8	***[c]
Originality of perception	1.6 ± 1.7	1.5 ± 1.2	
Hostility	1.6 ± 1.6	2.4 ± 1.7	***
Anxiety	3.9 ± 2.0	3.8 ± 2.2	
Bodily Preoccupation	0.4 ± 0.8	0.8 ± 1.1	*[b]

[a] Source: Hanninen et al., 1976

[b] Paired Chi Square-test for dichotomized scores

[c] Paired t-test

*P < 0.05; **P < 0.01; ***P < 0.001

reaction times were unaffected by exposure. It should be noted that in other studies, reaction time increased as a result of acute (Ogata et al., 1970; Gamberale and Hultengren, 1972) and subchronic (Wilson, 1973) exposures to toluene concentrations in excess of 200 ppm. The possible influence of differences in initial intelligence levels on the performance scores was controlled in the Hanninen et al. (1976) study by a separate comparison of the test results of 33 pairs of exposed and unexposed subjects who were matched for age and intelligence.

In a related study, Seppalainen et al. (1978) examined the same cohort of car painters studied by Hanninen and coworkers (1976) for neurophysiological effects. Results of EEG analysis on 102 solvent-exposed car painters and 102 nonexposed control subjects showed no increase in abnormalities (Abnormal EEGs were encountered in 32 painters and 37 controls.). It was noted, however, that the incidence of abnormal EEGs in both groups was higher than expected (approximately 10%) on the basis of EEG literature. It was further reported that 26 of the car painters had a complex of four subjective symptoms indicative of CNS disturbance (interrupted sleep, absentmindedness, easy to fall asleep when watching television, frequent headaches); this symptom complex was found only in 12 controls. EEG testing on the workers with these symptoms showed abnormalities in 46% (12/26) of the cases, but 26% (20/76) of those without the symptom complex also displayed EEG abnormalities. This difference was not statistically significant (Chi squared = 2.68).

Rouskova (1975) did observe changes in EEG response to photic stimulation in a group of 20 workers with a 13.5 year (average) history of exposure to higher concentrations of toluene (>250 ppm) and 1,1,1-trichloroethane (concentration not stated). Photic stimulation was applied in a series of rhythmic flashes, each lasting 10 seconds with intervals of 10 seconds between each flash series; frequencies ranged from 1 to 30 per second. Evaluated as a normal response was the occurrence of EEG activity of the same frequency as stimulation or of a harmonic or a subharmonic multiple of that frequency lasting at least one second. Results showed that abnormal EEG responses were found in 18 of the 20 workers (90%), but in only 1 of 20 unexposed control subjects.

Residual effects indicative of cerebellar and cerebral dysfunction have been observed in a number of persons who had abused toluene or solvent mixtures containing toluene over a period of years (Grabski, 1961; Satran and Dodson, 1963; Knox and Nelson, 1966; Kelly, 1975; Boor and Hurtig, 1977; Weisenberger, 1977; Keane, 1978; Sasa et al., 1978; Tarsh, 1979; Malm and Lying-Tunell, 1980; Metrick and Brenner, 1982). Boor and Hurtig (1977) described a case of cerebral involvement in an optician who regularly used toluene occupationally to clean eyeglasses and contact lenses in a small, unventilated room, and optic neuropathy has been observed (Keane, 1978; Malm and Lying-Tunell, 1980; Metrick and Brenner, 1982). Clinical signs in these individuals included ataxia, intention tremors, nystagmus, equilibrium disorders, positive Babinski reflex, impairment of speech and hearing, reduced vision, disturbance of concentration and memory, emotional lability, and psychosis. These reports, which are summarized in Table 11-6, indicate that the severity of the encephalopathic effects generally varied with the intensity and duration of exposure and that the effects were largely reversible, particularly when the exposures were not too extreme. Prolonged toluene abuse has, however, on occasion led to permanent encephalopathy and brain atrophy as evidenced by EEG and neuroradiological (pneumoencephalogram, angiogram) changes (Knox and Nelson, 1966; Boor and Hurtig, 1977; Sasa et al., 1978). Pontomedullary atrophy and abnormal brainstem auditory evoked potentials were recently observed in two chronic toluene abusers (Metrick and Brenner, 1982).

11.1.2. Peripheral Nervous System. Matsushita et al. (1975) found evidence of peripheral neuropathy in a group of 38 female shoemakers (mean age 20.7 ± 5.2 years) who had been exposed to a glue containing mainly toluene and "slight" gasoline for an average duration of 3 years and 4 months. The results of neurological and muscular function tests reportedly showed abnormal tendon reflexes, reduced grasping power of the dominant hand, and decreased finger tapping tempo in the exposed workers relative to a group of 16 unexposed control women (Table 11-7), but descriptions of the tests were not provided. A significant decrease in finger agility was also noted in the exposed shoemakers; agility of the fingers was estimated by measuring the time needed to move 25 "bulbs" using glass chopsticks. The average toluene concentration in the air varied with time of year from 60 to 100 ppm (range 15 to 200 ppm); in a "few" working places, gasoline ranged from 20 to 50 ppm. An increased urinary hippuric acid level among the exposed women (3.26 ± 0.82 mg/ml versus 0.35 ± 0.24 mg/ml for controls) is consistent with an exposure to toluene.

Electroneuromyographic measurements were made in the Seppalainen et al. (1978) study (described in Section 11.1.1.) on 59 of the toluene-exposed car-painters and 53 referents with a similar age distribution for any indication of a

TABLE 11-6

Encephalopathic Effects of Chronic Toluene Abuse

Subject (Age)	Exposure History	Effects and Diagnosis	Reference
Male (33 years)	Regularly sniffed toluene for 14 years. Subject purchased a gallon of pure toluene every 4-6 weeks, and inhaled the toluene on an almost daily basis at frequent intervals throughout the day.	Patient initially examined after 6 years by Grabski; signs included ataxia, intention tremors, pyramidal signs and psychosis which were concluded to be consistent with cerebellar degeneration. After 8 more years of abuse, Knox and Nelson reexamined the patient and concluded that the syndrome was primarily a diffuse cerebral disorder based on findings of ataxia, tremors, limb incoordination, emotional lability, marked snout reflex, and positive Babinski toe reflex; cerebral atrophy was confirmed by EEG and pneumoencephalography.	Grabski, 1961; Knox and Nelson, 1966
Male (30 years)	10-year history of toluene abuse.	Recurrent headaches, "inappropriate" speech, brief episodes of memory loss, increased irritability, and exaggerated swings in mood. Unremarkable clinical and neurological exam, but nonspecific EEG changes were found that were regarded as consistent with diffuse encephalopathy.	Satran and Dodson, 1963
Female (19 years)	Almost daily sessions of prolonged paint sniffing for 1-1/2 years. Ingredients not specified but it was indicated that toluene was a common ingredient in all the brands sniffed. Previous 4-year history of multiple drug and solvent abuse.	Ataxia, intention tremors of hands and feet, incoordination, hallucinations. Normal EEG, brain scan, arteriography, and pneumoencephalography. The diagnostic impression was cerebellar dysfunction secondary to some toxic factor in the paint. Objective neurological improvement 5 months after sniffing was discontinued.	Kelly, 1975
Male (25 years)	10-year history of lacquer thinner (99% toluene) abuse; during the last 5 years he had spent virtually all his waking hours inhaling the vapors (1 gallon used every 2 weeks)	Ataxia, mildly slurred speech, nystagmus, and bilateral Babinski signs. Normal EEG, nuclide brain scan, electromyogram, and nerve conduction studies, but a computerized brain scan showed diffuse widening of the cortical and cerebellar sulci. Subjective improvement in condition following abstinence from exposure, but a neurological exam after 9 months was essentially unchanged.	Boor and Hurtig, 1977

Table 11-6. (cont.)

Subject (Age)	Exposure History	Effects and Diagnosis	Reference
Male (59 years)	Optician who frequently but intermittently used 99% toluene in a small unventilated room to clean eyeglasses and contact lenses. Unable to smell toluene because of chronic anosmia. Duration of exposure not stated.	Fatigue and clumsiness of the left side which got progressively worse. Occasional staggering and mildly slurred speech, disturbed concentration and memory. Normal neurological exam, EEG, and brain scans. Daily improvement without specific treatment following cessation of exposure.	Boor and Hurtig, 1977
Male (age not stated)	Habitual inhalation of paint thinner (toluene) on the job. Duration not stated.	Bizzare behavior prior to hospital admission. Admitted in an agitated, violent, nearly catatonic state.	Weisenberger, 1977
Male (27 years)	Sniffed unspecified glues and paint thinners for 10 years. From age 25, toluene was involved 4-5 times per week (200-300 ml/week used), and from age 26, he inhaled 4-7 times per day (100 ml/day used.	Arm and neck tremors, ataxia, incoordination, and equilibrium disorders. No abnormal psychiatric symptoms. Pneumoencephalographic and angiographical evidence of midbrain and cerebrum atrophy. Degeneration of the cerebellum suspected.	Sasa et al., 1978
Male (20 years)	3-year history of daily aerosol spray paint inhalation. Product contained copper, toluene, and xylene as solvents and isobutane propane and methylene chloride as propellants.	Reduced vision, poor color perception, constricted visual fields, normal optic fundi, impaired papillary response, ataxia, and nystagmus. Symptoms slowly subsided following cessation of paint sniffing.	Keane, 1978
Male (25 years)	Sniffed toluene for 4 months, starting while on the job using toluene as a solvent in the rubber processing industry.	Delusions and unpredictable behavior. Largactil prescribed because he was thought to have a schizophrenic illness. Symptoms disappeared and did not recur following termination of sniffing.	Tarsh, 1979
Female (18 years)	Inhaled pure toluene since age 12, regularly since age 16 (2 liters used per month). Sniffed more heavily than usual during the last 2 months.	Personality changes (apathy, irritability, emotional lability, carelessness), vomiting, difficulty in walking, and slurred speech 1-2 weeks before admission. Gait ataxia, incoordination, dysarthria, downbeat nystagmus, bilateral positive Babinski sign, visual and color sense loss, impaired concentration and abstracting ability upon admission. Symptoms consistent with mainly cerebellar-brain stem involvement and possibly optic neuritis. Symptoms decreased when she did not inhale toluene, and disappeared after 8 months.	Malm and Lying-Tunell, 1980

TABLE 11-7

Results of Neurological and Muscular Function Tests of Toluene-Exposed Female Shoemakers[a]

Test[b]	Exposed Group	Control Group
Abnormal tendon reflex:		
Biceps and triceps	6(16)	3(19)
Patellar	14(37)*	1(6)
Ankle	7(18)**	0(0)
Pathological reflex	1(3)	0(0)
Grasping power (dominant hand)	11(29)**	1(6)
Tapping tempo (M ± S.D.)[c]	162.9 ± 16.6	168.6 ± 17.3
Cold pressure test	6(16)	2(13)
Postural hypotension	2(5)	1(6)
Cuff test (upper arm)	5(13)	1(6)
Dermatographism	5(13)	1(6)
Blocking test (M ± S.D.) (seconds)	68.2 ± 13.3	61.8 ± 13.7
Numbers investigated	38(100)	16(100)

[a] Source: Matsushita et al., 1975

[b] Numbers of subjects with abnormal scores reported. The percentage of subjects affected is indicated in the parentheses.

[c] Unit of measurement not stated.

Statistical significance (Chi Square- and t-tests): $^*P < 0.05$; $^{**}P < 0.1$; M = mean; SD = standard deviation.

possible peripheral neurotoxic effect from exposure. Maximum motor conduction velocity (MCV), conduction velocity of the slower motor fibers (CVSF), maximal sensory conduction velocity (SCV), and motor distal latencies were recorded from nerves in the upper and lower extremities (median, ulnar, deep peroneal, posterior tibial, and sural nerves). Results of these measurements showed that the mean conduction velocities and motor distal latencies of the car painters were almost identical to those recorded for the unexposed control group. In several instances, however, individual nerve conduction velocities were found to be slower than the normal historical value (not stated) for Seppalainen's laboratory. When the conduction velocities of the study group were compared with the historical values, abnormally slow MCVs or SCVs and/or prolonged motor distal latencies were found in 12 of the 59 painters, but in none of the 53 controls.

Although the two previous reports (Matsushita et al., 1975; Seppalainen et al., 1978) indicate a possible effect of toluene on the peripheral nervous system, toluene's role in the causation of human peripheral neuropathies has not been clarified. Reports of polyneuropathies in abusers exposed to excessive and prolonged concentrations of glues and solvents have appeared in the Japanese and American literature, but have in all cases involved mixtures of toluene and other solvents (Matsumura et al., 1972; Takenaka et al., 1972; Goto et al., 1974; Shirabe et al., 1974; Suzuki et al., 1974; Korobkin et al., 1975; Oh and Kim, 1976; Towfighi et al., 1976; Altenkirch et al., 1977). The cases described in these reports were characterized by the sudden onset and rapid progression of a symmetric, predominantly motor polyneuropathy (although sensory nerve involvement of the glove and stocking type has been reported), even after exposure has ceased. Symptoms included extremity weakness, numbness, paresthesia, marked amyotrophy, and occasional flaccid paresis. Collective results of electromyographic studies have shown delayed nerve conduction velocities and signs of denervation, and biopsies of nerves have revealed axonal degeneration, demyelination, and enlargement of some axons with focal accumulation of neurofilaments. Muscle biopsies revealed extensive neurogenic atrophy.

The earlier reports regarded either n-hexane alone (Korobkin et al., 1975; Towfighi et al., 1976) or a combination of n-hexane and toluene (Matsumura et al., 1972; Goto et al., 1974; Shirabe et al., 1974; Suzuki et al., 1974) as the cause of glue sniffers' neuropathy. The following observations have been offered as evidence to indicate that n-hexane plays an important role in its etiology: (1) in many of the reported cases, neuropathy did not develop until the patients began to sniff glue products that contained n-hexane, and (2) it is known that continuous occupational exposure to n-hexane under poor ventilation conditions produces a neuropathy among workers that is clinically and pathologically similar to that observed among the glue sniffers. From a recent outbreak of polyneuropathy among 18 glue thinner sniffers in West Germany, however, Altenkirch et al. (1977) presented data that implicate methyl ethyl ketone (MEK) as the causative agent and argue against n-hexane and toluene as the causes. These data are summarized as follows (Altenkirch et al., 1977):

1. In a number of sniffing adolescents (1000 to 2000), no adverse neurological effects were observed during the abuse of a thinner with a high n-hexane (31%) and toluene (30%) content over a period of 7 years.

2. The clinical picture of neuropathy occurred when the n-hexane fraction had been decreased by approximately one-half (16%) and MEK (11%) had been added; the amount of toluene was not significantly changed (29%).

3. Individuals who had discontinued sniffing prior to the introduction of the new formulation or who had used only the old composition were not affected. Neuropathies occurred, however, after 3 to 4 months in sniffers who had used only the new mixture.

4. Sniffing even a relatively small amount of the MEK-containing composition led to neurotoxic damages, while comparatively large amounts of the old composition were tolerated for a long time without consequences.

5. After the MEK-containing thinner was taken off the market, new cases of the disease were not observed.

Altenkirch and coworkers (1977) further noted that the exact composition of the glues that contained n-hexane and toluene cited in many of the aforementioned reports is incompletely characterized, and concluded that it remains open to question whether n-hexane was the sole causative agent in those cases. It should be emphasized that no report in which peripheral neuropathy is attributed to the inhalation of toluene alone was located in the literature. Further, no sensory or neuromuscular involvement was detected in a patient who experienced permanent cerebral dysfunction following prolonged inhalation of 99% pure toluene (Boor and Hurtig, 1977).

11.2. EFFECTS ON THE BLOOD AND HEMATOPOIETIC TISSUE

11.2.1. Bone Marrow. The action of toluene on human bone marrow has been the subject of persistent controversy. Early reports of occupational exposures (generally prior to the 1950s) ascribed myelotoxic effects to toluene (Ferguson et al., 1933; Greenburg et al., 1942; Wilson, 1943), but the majority of recent evidence indicates that the chemical is not toxic to the blood or bone marrow. The myelotoxic effects previously attributed to toluene are generally regarded by recent investigators to have been the result of concurrent exposure to benzene, which was present as a contaminant. Banfer (1961) noted that it first became possible to supply industry with adequate quantities of "pure" toluene ($\leq$0.3% benzene) in 1955; earlier, workers were typically exposed to toluene that was derived from coal tar and contaminated with as much as 20% benzene.

Greenburg et al. (1942) found mild depression of erythrocyte levels, absolute lymphocytosis, macrocytosis, and elevation of the hemoglobin level and the mean corpuscular hemoglobin concentration in 61 airplane painters who had been exposed to 100 to 1100 ppm toluene for periods extending from 2 weeks to 5 years (Table 11-8). Exposure was also associated with liver enlargement in 13 of the 61 painters (Section 11.3.), but not with abnormal granulocytic leukocyte counts, differential granulocytic leukocyte counts, reticulated erythrocyte counts, basophilic aggregation estimates, platelet counts, erythrocyte sedimentation rates, coagulation time, hematocrit values, erythrocyte fragility, or serum bilirubin levels. Approximately 75% of the painters were exposed to concentrations of 500 ppm or less, and the group had no known prior exposure to benzene. However, the contamination of the toluene vehicle in the paint with benzene cannot be precluded (NIOSH, 1973), because these blood changes are consistent with those of benzene poisoning. Volatile components such as ethyl alcohol, ethyl acetate, butyl alcohol, and petroleum naphtha were also present in quantity in the lacquers, dopes, and brushes used by the workers (Table 11-9).

In 1943, Wilson reported that of approximately 1000 industrial workers (industry not stated) exposed to 50 to 1500 ppm of commercial toluene vapor for 1

TABLE 11-8

Results of Blood Examinations Performed on Toluene-Exposed Airplane Painters[a,b]

	Toluene-Exposed Workers	Unexposed Workers
Erythrocytes counts $<5.2 \times 10^6/\text{mm}^3$	13.1% (N = 61)	5.2% (N = 346)
Lymphocytes counts $>5000/\text{mm}^3$	20.4% (N = 59)	7.7% (N = 395)
Mean Corpuscular Volume $\geq 100\ \mu^3$	21.3% (N = 61)	7.2% (N = 111)
Hemoglobin $\geq$16g/100 mℓ	29.5% (N = 61)	2.4% (N = 81)
Mean Corpuscular Hemoglobin $\geq$35 picograms	13.1% (N = 61)	0% (N = 73)
Mean Corpuscular Hemoglobin Concentration % of cases $\geq$35g/100 mℓ	34.4% (N = 61)	2.5% (N = 81)

[a] Source: Greenburg et al., 1942

[b] Percent abnormal cases reported.

TABLE 11-9

Analysis of Paint Used by Painters[a]

	Percentage in Mixture
Spray painters	
Primer (75% of paint used):	
Zinc chromate	10.8
Magnesium silicate	0.7
Synthetic resin	12.8
Driers (lead and cobalt compounds)	0.3
Xylene	5.8
Toluene	69.6
	100.0
Lacquer 1 (15% of paint used):	
Volatile portion:	
Ethyl alcohol	7.0
Ethyl acetate	18.0
Butyl alcohol	7.0
Butyl acetate	15.0
Petroleum naphtha	3.0
Toluene	50.0
	100.0
Nonvolatile:	
Nitrocellulose, synthetic resin, titanium oxide, ferrocyanide blue, iron oxide, carbon black, zinc oxide, etc. No lead compounds	
Lacquer 2 (10% of paint used):	
Volatile portion:	
Toluene	25.0
Xylene	33.0
Petroleum naphtha	42.0
	100.0
Nonvolatile:	
Resin, titanium oxide, zinc oxide, ultramarine blue, ferrocyanide blue, iron oxide, diatomaceous earth, amorphous silica, carbon black	

TABLE 11-9 (cont.)

	Percentage in Mixture
Brush painters	
Dope:	
Volatile portion:	
Ethyl acetate	
Ethyl alcohol	16.5
Butyl acetate	3.2
Butyl alcohol	16.5
Petroleum naphtha	5.6
Toluene	13.7
	44.5
	100.0
Nonvolatile:	
Nitrocellulose, glycol sebacate, aluminum, cadmium sulfide, barium sulfate	
Brush wash:	
Acetone	
Ethyl alcohol	22.5
Toluene	22.5
	55.0
	100.0

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[a] Greenburg et al., 1942

[b] Dip painters used a primer only of the same composition as given for spray painters.

to 3 weeks, 100 showed symptoms attributable to toluene intoxication. Ten of the 100 workers had been exposed to concentrations in excess of 500 ppm and showed signs of serious CNS toxicity (Section 11.1.1.2.). In most of these 10 cases, all blood elements remained normal except for the red cell count, which was "usually" reduced ($\approx 2.5 \times 10^6/mm^3$). In 2 of the 10 cases, leukocytes (2500 to $3000/mm^3$) and platelets were reduced as well, and differential counts showed decreased polymorphonuclear cells and reticulocytes, and increased monocytes. Sternal bone marrow biopsies in these two cases showed partial degeneration of the blood-forming elements, which resulted in a diagnosis of aplastic anemia. No clinical blood changes were seen in the workers who had been exposed to the lower concentrations of toluene (i.e., <500 ppm).

Von Oettingen et al. (1942a, 1942b) were the first workers to document the effects of essentially pure toluene on human subjects. The toluene used was shown, on spectrophotometric analysis, to contain not more than 0.01% benzene. In this study, no significant changes in the total or differential white cell count were found in 3 volunteers following controlled 8-hour exposures to various concentrations of toluene within the range of 50 to 800 ppm. Not more than 2 exposure sessions were performed per week to provide sufficient time for recovery between exposures, and the experiments were conducted over a period of 8 weeks (Section 11.1.1.1.). Erythrocyte counts were not made.

Parmeggiani and Sassi (1954) concluded from a clinical study of 11 paint and pharmaceutical workers exposed to 200 to 800 ppm toluene and 13 others with exposure to a combination of toluene (150 to 1900 ppm) and butyl acetate (150 to 2400 ppm) that toluene had no particular injurious action on the bone marrow (or other organs). The English summary of this study indicated that the workers were exposed for "many" years, but the purity of the toluene was not reported. Among the workers in the two groups, 34% reportedly showed slight anemia (<4,000,000 erythrocytes/mm^3), 15% had a mild neutropenia (<3500/mm^3), 26% were lymphocytotic (>2000/mm^3), and 45% showed a decrease in blood platelets (<150,000/mm^3) not accompanied by evident signs of capillary fragility.

In a more recent investigation, Banfer (1961) examined 889 rotogravure printers and helpers who were exposed to the vapors of toluene-containing printing inks for at least 3 years. Four hundred and seventy eight non-exposed persons from two groups served as controls; one group was composed of 155 management workers from the same plant, and the second group was composed of 323 persons from outside the plant. The available commercial toluene used in these inks reportedly contained only traces of benzene (<0.3%); when 5 samples of the toluene were examined by Banfer, no traces of benzene were found, but the method of analysis and detection limits were not stated. Analysis of the room air for toluene was performed by infrared spectroscopy, but limited to 5 samples taken from different sites on a single day. Ambient toluene concentrations were not specified, but three of the samples were determined to be below the "MAK-Wert," the fourth sample was at the "MAK-Wert," and the fifth sample, taken near one of the presses, exceeded the "MAK-Wert" by 400 ppm. A translation of this study by NIOSH (1973) indicates that the "MAK-Wert" was 200 ppm. Hematologic examinations of the workers and controls did not reveal any significant changes in the total number of leukocytes, lymphocytes, granulocytes, or erythrocytes, or hemoglobin levels (Table 11-10). Sternal biopsies from 6 printers with white cell counts of less than $5000/\text{mm}^3$ were normal.

TABLE 11-10

Hematologic Examination of 889 Rotogravure Workers[a]

	Printers (N = 889)	Controls, Group 1[b] (N = 155)	Controls, Group 2[c] (N = 323)
Erythrocytes			
counts $<4 \times 10^6/\text{mm}^3$	16 (1.79%)	3 (1.93%)	7 (2.10%)
Leukocytes, total			
counts $> 8500/\text{mm}^3$	78 (8.77%)	11 (7.09%)	26 (8.04%)
counts $<5000/\text{mm}^3$	74 (8.32%)	18 (11.61%)	38 (11.76%)
counts $<4500/\text{mm}^3$	28 (3.15%)	4 (2.58)	12 (3.71%)
counts $<4000/\text{mm}^3$	3 (0.33%)	1 (0.64%)	1 (0.30%)
Lymphocytes			
<35% total leukocytes	25 (2.81%)	3 (4.16%)	4 (1.32%)
total counts $<5000/\text{mm}^3$	889 (100%)	155 (100%)	323 (100%)
Granulocytes			
total counts $>2000/\text{mm}^3$	889 (100%)	155 (100%)	323 (100%)
Hemoglobin			
value <13g/100mℓ	4 (0.45%)	4 (2.58%)	4 (1.23%)

[a] Source: Banfer, 1961

[b] Unexposed management workers from the same plant

[c] Unexposed individuals not employed at the plant

Capellini and Alessio (1971) performed hematological examinations on 17 workers who had been exposed for "diverse" years to 125 ppm toluene (range, 80 to 160 ppm) in a plant manufacturing V-belts for industrial machinery. Results showed that the hemoglobin values, red cell counts, white cell counts, and platelet counts of the workers were within the same limits as those of 19 non-exposed control subjects from the same plant. The benzene content of the toluene was not reported. Blood findings were also within normal limits in another worker employed in a different department who was exposed to mean toluene concentrations of 250 ppm (range, 210 to 300 ppm) and who demonstrated symptoms of CNS toxicity and conjunctival irritation.

In 1975, a report by the West German Association of Gravure Printers (Suhr, 1975) identified a study population of 100 printers with at least 10 years of exposure to pure toluene (<0.3% benzene) and an unexposed control group of equal size from the same plant. Analysis of air samples collected from the workplace reportedly indicated that the potential exposure to toluene ranged from 200 to 400 ppm. Blood analyses (hemoglobin, erythrocyte, leukocyte, thrombocytes, differential analysis) demonstrated no unusual frequency of abnormalities in either the exposed or control groups.

Matsushita et al. (1975) found no alterations in the specific gravity of whole blood, hemoglobin content, hematocrit, or white blood cell counts in a group of 38 female shoemakers who had been exposed to toluene (60 to 100 ppm average) and, in a "few" working places, gasoline (range, 20 to 50 ppm) for an average duration of 3 years and 4 months. The hematological test results from the shoemakers were compared with those from an unexposed control group of 16 female workers. A significantly increased number of "Mommsen's" toxic granules were observed in the neutrophils of the exposed workers. Thirteen of the 38 workers showed an abnormal appearance of the granules (mean number per neutrophil, 7.6 ± 5.6) compared with 1 of 16 controls (mean number per neutrophil, 3.8 ± 3.4).

Further evidence of the relative non-toxicity of toluene to the hematopoietic system was presented by Francone and Braier (1954). Toluene, because of its presumed myelotoxic action, was administered orally as a treatment for leukemia. It was found that daily doses of up to 10 g of toluene in olive oil for 3 weeks (to a total of 130 g) were tolerated by leukemia patients without complaints or evidence of side effects, but the treatment had no clinical effect on the leukemic process.

Hematological abnormalities have been infrequently reported in sniffers of toluene-based glues. In a total of 90 cases surveyed by 4 groups of investigators (Christiansson and Karlsson, 1957; Massengale et al., 1963; Barman et al., 1964; Press and Done, 1967b), there were no instances of anemia or lymphopenia, a single report of neutropenia, and 6 cases of eosinophilia of greater than 5%. Christiansson and Karlsson (1957) also performed bone marrow examinations on 17 individuals; 10 of these showed changes suggestive of disturbances in maturation of leukocytes, although these changes were not reflected in the peripheral blood of the same individuals. The individuals examined in the Christiansson and Karlsson (1957) study were habituated to the inhalation of toluene-based paint thinners, rather than model glues as were the subjects in other surveys. In a fifth clinical survey of 89 glue sniffers, however, Sokol and Robinson (1963) found abnormalities of the blood in 68 of the cases. An effect on the white blood cells was indicated by findings of eosinophilia (25 subjects), leukocytosis (12 cases), and lymphopenia (4 subjects). Sokol and Robinson (1963) also reported low hemoglobin values in 20 subjects and basophilic stippling of erythrocytes in 42 of the patients, and noted the frequent occurrence of poikilocytosis (25 cases), anisocytosis (20 cases), hypochromia (14 cases), and polychromasia (10 cases). There is no obvious explanation for the discrepancy between the hematological findings of Sokol and Robinson (1963) and those of the other investigators. However, since none of the aforementioned cases deal with exposure to pure toluene, the abnormalities observed should be considered to be the possible result of contamination of the toluene by benzene or some other organic solvent.

Powars (1965) diagnosed five cases of acute aplastic anemia that were associated with glue sniffing in black adolescents with pre-existing sickle-cell disease. The 5 children had apparently used 3 different glues, 2 containing toluene and 1 containing acetone. All of these patients recovered following transfusion and cessation of sniffing. A case of fatal aplastic anemia, uncomplicated by the presence of sickle-cell disease, was described in a sixth individual with a 3-year history of glue sniffing.

11.2.2. Blood Coagulation. Pacseri and Emszt (1970; cited in NIOSH, 1973) reported that an increase in prothrombin time was found in 191 printers exposed to 170 to 340 ppm toluene (duration of exposure not stated). Two of the subjects showed a reduced number of red blood cells, but no other hematologic abnormalities were found in these workers. The benzene content of the toluene was not reported.

11.2.3. Phagocytic Activity of Leukocytes. It has been reported that the phagocytic activity of leukocytes from printing-plant workers exposed to toluene vapors was significantly reduced relative to a control population (Bansagi, 1968). There was no relationship, however, between the decrease in activity and the concentration of toluene in the air. The Chemical Abstracts summary of this Hungarian study did not detail any of the exposure information or mention the benzene content of the toluene.

Friborska (1973; cited in NRC, 1980) noted increased concentrations of alkaline phosphatase and lactic acid dehydrogenase in leukocytes and increased acid phosphatase in both leukocytes and lymphocytes from workers who were routinely exposed to toluene. The authors associated these alterations with increased functional capacity of the cells.

11.2.4. Immunocompetence. Serum immunoglobulin level (Lange et al., 1973a) and leukocyte agglutinins (Lange et al., 1973b) were studied in a group of 35 workers with a history of exposure to benzene, toluene, and xylene. The duration of exposure ranged from 1 to 21 years and the concentration of these compounds in the air ranged from 0.011 to 0.17 mg/ℓ, 0.08 to 0.23 mg/ℓ, and 0.12 to 3.0 mg/ℓ, respectively. Serum IgG and IgA levels were found to be significantly lower in the solvent-exposed workers than in non-exposed controls, although IgM levels tended to increase (Lange et al., 1973a). Lange and coworkers (1973b) also found that 10 of the 35 workers had leukocyte agglutinins for autologous leukocytes, and demonstrated an increase of leukoagglutination titer in human sera after incubation with benzene, toluene or xylene; this suggested that some workers exposed simultaneously to these aromatic compounds may exhibit allergic blood dyscrasias. In another group of workers (N = 79) with a similar history of exposure to benzene, toluene, and xylene (i.e., levels and durations of exposure comparable to those of the workers examined by Lange et al.), Smolik et al. (1973) found a decreased level of serum complement. It should be noted that in all of the aforementioned studies, the specific solvent(s) responsible for the changes were not identified.

11.3. EFFECTS ON THE LIVER

Greenberg et al. (1942) found enlarged livers in 13 of 61 airplane painters (21%) who were exposed to 100 to 1100 ppm toluene for 2 weeks to more than 5 years. Toluene was the major solvent used in the paints, although significant quantities of other volatile components were present (Table 11-9); these workers reportedly had no history of inhalation exposure to any other toxic volatile solvents, including benzene. This incidence of liver enlargement was 3 times that observed in a control group of 430 workers who had never been exposed to toluene, but it cannot be correlated with exposure level, because only the numbers of workers exposed at different exposure levels (and not hepatomegaly incidences) were reported. The liver enlargement was diagnosed by palpitation, and in no cases were the livers tender. There was also no correlation between the enlarged livers and either clinical or laboratory (blood and urine analyses) evidence of disease, and it was suggested that the enlargement might have been compensatory in nature.

Greenburg and coworkers' (1942) finding of hepatomegaly has not been substantiated in subsequent studies of workers with histories of occupational toluene exposure. Parmeggiani and Sassi (1954) found a comparable incidence (27%) of enlarged livers in a group of 11 paint and pharmaceutical production workers who were exposed to 200 to 800 ppm toluene for "many" years, and in a control group of unexposed workers from the same plant. Normal liver function, as determined by electrophoresis, serum colloid stability testing, and galactose tolerance testing, was also observed in the exposed workers. Capellini and Alessio (1971) observed no changes in "the function of the liver" in 17 workers exposed for "diverse" years to a mean atmospheric concentration of 125 ppm toluene (range, 80 to 160 ppm) in a plant manufacturing V-belts for industrial machinery. Liver function in this study was evaluated by determinations of total serum protein and protein electrophoresis.

More recently, Suhr (1975) similarly found comparable, but high, incidences of enlarged livers and elevated liver enzymes in a group of 100 gravure printers with at least 10 years' exposure to 200 to 400 ppm pure toluene (benzene $\leq 0.3\%$), and in a control group of 100 workers from the same company who had not been exposed to toluene. It should be noted that the nature and history of the control group was not defined in any greater detail. Enlargement of the liver was established in 22% of the printers and 20% of the control group, and liver enzyme assays showed that about half of all test persons (50% of the printers, 51% of the controls) had increases in serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), glutamic dehydrogenase (GLDH), or gamma glutamyl transferase (GGT) levels. It was concluded that because of the equal distribution of affected persons in both groups, the deviations in these parameters could not be attributed to toluene exposure. The cause of the hepatomegaly and liver enzyme deviations was not further investigated. Blood alcohol determinations before and after the workshifts indicated comparably elevated levels in both the printers and control group, but less than half of the 100 subjects in each group were tested; approximately half of the tested subjects had levels between 0.01 and 0.1%. The significance of the elevated blood alcohol levels is unclear, however, because of the small number of subjects tested, because only single blood alcohol determinations were performed on each subject, and because the data were presented ambiguously.

Other studies have reported significant effects on indices of liver function in groups of toluene-exposed workers. In an examination of 94 rotogravure printers with a history of exposure to 18 to 500 ppm toluene and of a reference group of 30 municipal clerks, Szadkowski et al. (1976) found significant reduction in bilirubin and alkaline phosphatase in the exposed group, but no difference from controls in SGOT, SGPT, leucinamino-peptidase, or cholinesterase levels. The 94 rotogravure workers were categorized into four groups by intensity of exposure to toluene. The mean exposure levels, durations of exposure and ages of the groups were, respectively (Szadkowski et al., 1973): Group 1 (N = 68) - 300 ppm, 7.3 ± 5.3 years, 32 years; Group 2 (N = 4) - 426 ppm, newly appointed on day of investigation, 24.3 years; Group 3 (N = 11) - 82 ppm, 5.6 ± 5.2 years, 42.9 years; Group 4 (N = 11) - 18 ppm, 8.5 ± 4.4 years, 35.8 years. Blood alcohol levels ranged from 0.02% to 0.07% in the exposed workers.

Trevisan and Chiesura (1978) performed the following hepatic function tests on 47 subjects who were exposed occupationally to toluene via inhalation: bilirubin, SGOT, GGT, alkaline phosphatase (AP), ornithine-carbamyl transferase (OCT), Quick's test, and protein measurement. All tests gave normal results with the exception of GGT, which was reportedly above normal (28 μ/mℓ) in 34% of the cases. In a group of 12 subjects controlled before and after toluene entered in the working operation, mean GGT activity increased 2-fold after exposure. Although GGT has proved to be a very sensitive screening enzyme for slight changes in liver function (Dragosics et al., 1976), it should be noted that the data from this study were published in abstract form, and that information on exposure or type of occupation and detailed results of the hepatic function tests were not presented.

The mean serum activities of four liver enzymes (aspartate transaminase, alanine aminotransferase, OCT, GGT) did not differ between a group of 102 car painters who were exposed to a mixture of organic solvents, and an age-matched unexposed reference group of 102 men (Kurppa and Husman, 1982). The exposed subjects were exposed mostly to low levels of toluene (30.6 ppm detailed in Table 11-3) for 1 t0 40 years (mean, 14.8 + 8.5 yrs), and the age of the painters ranged from 20 t0 65 (mean 35 ± 11 yrs). Abnormal intellectual/psychomotor performance (Hanninen et al. 1976), abnormal neurophysiological effects (Seppalainen et al., 1978) and an increased frequency of lens changes (Raitta et al., 1976), however, have been observed in these workers. Abnormally slow motor and sensory conduction velocities and/or prolonged motor digital latencies were suggested in 12 of 59 car painters studied (Section 11.1.2), and ophthalmological examination revealed lens opacities in 48 of 92 car painters (Section 11.7.1). Kurppa and Husman (1982) found that the liver enzyme activities of these two "solvent-affected" subgroups were similar to those of the car painters with no corresponding abnormalities.

English summaries of two Polish studies of women with histories of occupational exposure to toluene indicated abnormalities in the glycoprotein, serum mucoid and haptoglobin patterns of 53 women (Kowal-Gierczak et al., 1969), and changes in the serum levels of iron and copper, and urinary excretion of porphyrin in 51 women (Cieslinska et al., 1969). Although these changes may be indicative of liver dysfunction, clinical signs of liver function impairment were not observed in these subjects. The concentrations of toluene, durations of exposure, and the possibility of exposure to other chemicals were not discussed in the available summaries.

Intensive exposure to toluene via glue or thinner sniffing appears to have a minimal effect on the liver. Results of hepatic function tests (SGOT, SGPT, AP, bilirubin, sodium sulfobromophthalein excretion, serum proteins, cephalin flocculation) on a total of 179 sniffers who were examined in early clinical surveys were essentially unremarkable (Christiansson and Karlsson, 1957; Massengale et al., 1963; Sokol and Robinson, 1963; Barman et al., 1964; Press and Done, 1967a, 1967b). Christiansson and Karlsson (1957) apparently did detect liver enlargement in 5 out of 32 Swedish lacquer thinner sniffers, but other signs of liver function were normal. More recently, Litt and coworkers (1972) found elevated SGPT and AP levels in 2 and 5%, respectively, of a group of 982 glue sniffers.

Grabski (1961) described an individual who had abused pure toluene for six years and showed signs of cerebellar degeneration, hepatomegaly, and impaired liver function. Complete series of liver function tests were normal, however, in an optometrist and a glue sniffer exposed independently to 99% pure toluene, both of whom also exhibited encephalopathic effects (Boor and Hurtig, 1977). Reversible hepatorenal damage was diagnosed in an individual with a 3-year history of inhaling a cleaning fluid that contained 80% toluene (other components not known) coupled with alcohol ingestion (O'Brien et al, 1971); the hepatic effect was indicated by elevated serum bilirubin and AP.

11.4. EFFECTS ON THE KIDNEYS

Urinary findings were normal in 91 specimens from a group of 61 airplane painters (number of donors not stated) who were exposed to 100 to 1100 ppm toluene for 2 weeks to 5 years (Greenburg et al., 1942). Urinalysis consisted of specific gravity, albumin, and sugar determinations, and examinations for formed elements. Exposure to mean concentrations of 60 to 100 ppm toluene and 20 to 50 ppm gasoline in a "few" working places for an average duration of 3 years and 4 months did not result in abnormal urinalysis findings as determined by standard methods (protein, sugar, urobilinogen, bilirubin, occluded blood, keton body), except for excretion of hippuric acid, in 38 female shoemakers (Matsushita et al., 1975). Glomerular filtration rate (as measured by ^{51}Cr-EDTA clearance from plasma) was not reduced in a group of 34 rotogravure workers when compared with 48 non-exposed male controls (Askergren et al., 1981), but the toluene exposures were not characterized. Proteinuria and hematuria were noted, however, in a worker who was exposed to concentrations of toluene sufficient to cause unconsciousness while cleaning the inside of a tank that was coated with an emulsion of 45% toluene and 27% DDT (Lurie, 1949).

Reisin and coworkers (1975) published a report regarding the development of severe myoglobinuria and non-oliguric acute renal failure in a paint factory laborer who was exposed to pure toluene by skin contact and aspiration when a hose burst. The patient had inhaled sufficient amounts of toluene to cause a loss of consciousness for 18 hours and subsequent development of chemical pneumonitis and sustained superficial burns on approximately 10% of his body surface area. Acute renal failure apparently developed from the lack of fluid intake accompanied by heavy myoglobinuria rather than from a direct effect of toluene. The early administration of intravenous fluids and diuretics, and the use of hemodialysis led to complete recovery.

Pyuria, hematuria, and proteinuria have been the most frequently observed signs of renal dysfunction associated with the deliberate inhalation of toluene-based glues (Christiansson and Karlsson, 1957; Massengale et al., 1963; Sokol and Robinson, 1963; Barman et al., 1964; Press and Done, 1967a, 1967b). The clinical findings observed in 159 cases surveyed between 1957 and 1967 are tabulated in Table 11-11. These indications of renal dysfunction have not been universally observed in glue sniffers, are generally transient, and follow closely the intensive exposures (Press and Done, 1967b).

O'Brien et al. (1971) more recently described a case of reversible hepatorenal damage in a 19-year-old male who had a 3-year history of glue sniffing while employed in the sign-painting trade. Prior to hospital admission, the subject had spent 6 hours inhaling a cleaning fluid that contained 80% toluene (the other components were not identified). Upon admission, the patient was vomiting and anuric, and after 8 hours, periorbital edema and subconjunctival hemorrhages developed. Blood concentration of toluene was determined to be 160 ppm. In addition to diminished urine output, evidence of renal damage included hematuria, proteinuria, and elevated serum creatinine. The effects of these exposures on hepatic function are discussed in Section 11.3. (Effects on the Liver).

Although serious involvement of the kidney with human intoxication by toluene has not been stressed in the early literature, several reports have recently appeared that associate deliberate inhalation of toluene with metabolic acidosis (Taher et al., 1974; Fischman and Oster, 1979a; Kroeger et al., 1980; Bennett and Forman, 1980; Moss et al., 1980). The cases of acidosis described by these investigators (Table 11-12) are characterized by serious electrolyte abnormalities (hypokalemia, hyperchloremia), and are related primarily to toluene's ability to impair hydrogen ion secretion in the distal renal tubule (distal renal tubular acidosis). In addition to findings compatible with distal renal tubule acidosis, Moss et al. (1980) found pathologically increased excretion of amino acids, glucose, phosphate, uric acid, and calcium that indicated proximal tubule dysfunction consistent with Fanconi's syndrome. Kroeger et al. (1980) reported the case of a patient with toluene-induced renal tubular acidosis who developed recurrent urinary calculi. It should be noted that each of the subjects who developed acidosis had a history of multiple toluene abuse and, although the acute consquences of renal tubular acidosis associated with toluene sniffing were on occasion life threatening, these effects were completely reversible with abstinence from toluene exposure. These symptoms also responded promptly to electrolyte repletion therapy with potassium chloride and sodium bicarbonate.

TABLE 11-11

Renal Function Investigations of Glue Sniffers[a,b]

Number of Patients	Pyuria	Hematuria	Proteinuria	Clearances	Azotemia	Reference
32	All 32 urine samples "normal"; details not given	ND	ND	ND	ND	Christiansson and Karlsson, 1957
27	0	2	0	ND	0	Massengale et al., 1963
89[c]	32	14	12	ND	ND	Sokol and Robinson, 1963
15	0	0	1	PSP[d] 0/13	0/7	Barman et al., 1964
16	6	3	5/13	Urea 1/7	0/9	Press and Done, 1967b

[a] Source: Press and Done, 1967b

[b] Exposures were to toluene-containing plastic cements except in the Christiansson and Karlsson (1957) study, in which the subjects examined had sniffed paint thinner.

[c] Urinary abnormalities were found in 67 of the 89 glue sniffers.

[d] Phenosulfonphthalein clearance in 2 hours.

ND = not determined

TABLE 11-12

Toluene Induced Metabolic Acidosis

Subject (Age)	Exposure History	Symptoms	Clinical Findings	Reference
Male (23 yr)	Sniffed glue and pure toluene intermittently for 6 yr.	Several episodes of muscle weakness following prolonged (e.g., 4 to 7 d) inhalation sessions. One instance of flaccid paralysis.	Hypokalemia with hyperchloremic metabolic acidosis. Elevated urinary pH. Toluene detected in blood.	Taher et al., 1974
Female (20 yr)	Two 3 to 5 d episodes of sniffing aerosol paint containing 60% toluene within 4 wk.	Nausea.	Hyperchloremic acidosis. Elevated urinary pH. Toluene detected in blood.	Taher et al., 1974
Female (17 yr)	Sniffed transmission fluid containing 100% toluene for 5 d.[a]	Persistent vomiting.	High anion gap[b] metabolic acidosis.	Fischman and Oster, 1979a
Female (21 yr)	Intermittently sniffed transmission fluid containing 100% toluene for at least 5 yr.[a]	Hospitalized on 6 occasions within a 16 mo. period. Severe weight loss (18 kg) at first admission. Recurrent symptoms of vomiting, muscle weakness, and lethargy. After the 6th episode, patient died of cardiopulmonary arrest.	Hypokalemia. Hyperchloremic metabolic acidosis and high urinary pH on 1st and 6th admissions. High anion gap metabolic acidosis on the other admissions.	Fischman and Oster, 1979a
Female (25 yr)	Frequent sniffing of transmission fluid containing 100% toluene during a 5 yr period.[a]	Persistent vomiting, lethargy, and muscle weakness.	Normal anion gap hyperchloremic metabolic acidosis with severe hypokalemia.	Fischman and Oster, 1979a
Male (23 yr)	Sniffed toluene on a "regular" basis for 5 yr. Form not specified.	Hospitalized 4 times within 15 mo. History of vomiting, flank pain, and paralysis of the lower extremities.	Recurrent uretal and renal calculi (4 stones total). Hyperchloremic metabolic acidosis and hypokalemia. Acidic urine.	Kroeger et al., 1980
Female (27 yr)	Daily inhalation of glue for 9 mo.	Lethargy, weakness, and ataxia. Microscopic hematuria and sterile pyuria.	Hyperchloremic metabolic acidosis, hypokalemia, hypocalcemia, hypophosphatemia and hypouricemia. Increased excretion of 14 amino acids and glucose.	Moss et al., 1980
Four individuals (ages and sexes not stated)	Glue or paint sniffers (details not stated).	Not stated.	Hyperchloremic metabolic acidosis with hypobicarbonatemia.	Moss et al., 1980
Male (22 yr)	Abused a lacquer thinner (99% toluene) for 8 yr.	Abdominal pain, vomiting, generalized weakness, and diminished reflexes.	Hypokalemic and hypochloremic metabolic acidosis.	Bennett and Forman, 1980

[a] Toluene is not ordinarily a component of transmission fluid (Fischman and Oster, 1979b).

[b] Anion gap is defined as serum Na - (Cl + HCO_3) in milliequivalents per liter.

yr = year; d = day; wk = week; mo. = month

Fischman and Oster (1979a) found a high anion gap metabolic acidosis with hypokalemia in two patients who had sniffed 100% toluene; this condition is reportedly indicative of an increased production of acid by the body. Although it was noted that renal failure, ketonemia, and elevated lactate levels could have accounted in part for the abnormal increases in anion gap, it was suggested that the acid metabolites of toluene (e.g., benzoic and hippuric acids) may have caused the high anion gap metabolic acidosis.

Clinical manifestations associated with the reported metabolic alterations included nausea, lethargy, ataxia, muscular weakness, and paralysis (Table 11-12). The National Research Council has noted that some of these manifestations may mimic those usually attributed to the effects of toluene on the CNS, and that altered pH and electrolyte balance may be more commonly responsible for the manifestations of toluene abuse than is usually recognized (NRC, 1980). In particular, hypokalemia often produces significant muscular weakness including flaccid paralysis.

11.5. EFFECTS ON THE HEART

Ogata et al. (1970) found an apparent decrease in the pulse rate of 23 volunteers who were exposed to 200 ppm toluene for periods of 3 hours, or for 3 hours and a 1-hour break period followed by 4 additional hours, but no effect at 100 ppm. Systolic and diastolic blood pressure were not affected by exposure. Exposure to 100 and 200 ppm toluene for 30 minutes did not, however, have any effect on the heart rates or electrocardiograms of 15 other subjects during either rest or light exercise (Astrand et al., 1972). Other studies have shown that experimental exposure to toluene at levels of 100 to 700 ppm for 20 minutes (Gamberale and Hultengren, 1972) or 50 to 800 ppm for 8 hours (Von Oettingen et al., 1942a, 1942b) did not cause any definite effects on heart rate or blood pressure. Suhr (1975) noted that the pulse rates and blood pressures of a group of 100 printers with a 10-year history of exposure to 200 to 400 ppm toluene and those of an unexposed control group of identical size were similar at the beginning and end of work shifts.

Sudden deaths that were not due to suffocation secondary to solvent sniffing, but rather were attributed to a direct effect of the solvent itself have been reported in at least 122 cases (Bass, 1970; Alha et al., 1973). Toluene, benzene, and gasoline have been individually implicated in a small number of these deaths (10, 6, and 4 cases, respectively), but the volatile hydrocarbons most frequently involved were trichloroethane and fluorinated aerosol propellants. Severe cardiac arrhythmia resulting from light plane anesthesia was offered as the most likely explanation for the cause of the sudden sniffing deaths. Bass et al. (1970) noted that stress, vigorous activity, and hypoxia, in combination with sniffing, appear to increase the risk of death.

11.6. EFFECTS ON MENSTRUATION

Subjective complaints of dysmenorrhea were reported by 19 out of 38 Japanese female shoemakers (mean age, 20.7 years) who were exposed to mean toluene concentrations of 60 to 100 ppm for an average duration of 3 years and 4 months (Matsushita et al., 1975). In an unexposed control group of 16 women from the same plant, this effect was noted by 3 individuals (19%). It should be noted that these women were concomitantly exposed to 20 to 50 ppm of gasoline in a "few" working places. In this study, the presence or absence of 15 subjective symptoms was ascertained by questionnaire; in addition to dysmenorrhea, a significant number of workers reported "uneasy feelings" about the solvent vapor, and itching and dermatitis of the hands.

Michon (1965) reported disturbances of menstruation in a group of 500 women (age 20 to 40 years) who had been exposed to a mixture of benzene, toluene, and xylene in the air of a leather and rubber shoe factory. The concentration and component distribution of this mixture were not specified, but it was stated in the English summary of this Polish study to be within permissible occupational limits established at the time in Poland, 31 ppm (100 mg/m^3) for benzene, 67 ppm (250 mg/m^3) for toluene, and 58 ppm (250 mg/m^3) for xylene). When the menstrual cycles of the exposed women were compared with those of 100 women from the same plant with no exposure to these hydrocarbons, prolonged and more intense menstrual bleeding was reportedly found in the exposed group. The regularity of the cycle was not affected.

It has also been noted in the English summary of a Russian study that occupational exposure to average concentrations of 6 to 93 ppm (25 to 350 mg/m^3) toluene and other solvents, through the use of organosiliceous varnishes in the manufacture of electric insulation materials, caused a high percentage of menstrual disorders (Syrovadko, 1977). The newborn of these women were reportedly more often underweight and experienced more frequent fetal asphyxia and "belated" onset of nursing. Although the above studies suggest that occupational exposure to aromatic hydrocarbons may be associated with menstrual disturbances, it should be emphasized that a specific effect of toluene could not be determined from the available data. Information on the possible reproductive effects of toluene in males is not available.

11.7. EFFECTS ON THE RESPIRATORY TRACT AND THE EYES

11.7.1. Effects of Exposure. Carpenter et al. (1944) observed that 2 male subjects who were exposed to toluene for 7 to 8 hours experienced transitory mild throat and eye irritation at 200 ppm, and lacrimation at 400 ppm. Parmeggiani and Sassi (1954) found irritation of the upper respiratory tract and conjunctiva in 1 of 11 paint and pharmaceutical product workers who were exposed to 200 to 800 ppm toluene for "many" years. In the studies of Von Oettingen et al. (1942a,b) and Wilson (1943), however, no complaints of respiratory tract discomfort were recorded in volunteers or workers exposed to levels of toluene as high as 800 to 1500 ppm for 8-hour periods (Section 11.1., Effects on the Nervous System). In 2 episodes of accidental poisoning on ships that involved estimated short-term exposures to 10,000 to 30,000 ppm toluene, Longley et al. (1967) recorded no complaints of respiratory tract or eye irritation among 26 men.

Transient epithelial injury to the eyes that consisted of moderate conjunctival irritation and corneal damage, with no loss of vision, was observed in three workers who were accidentally splashed with toluene (McLaughlin, 1946; Grant, 1962). Complete recovery generally occurred within 48 hours. The results of opthalmologic examinations of 26 spray painters who were exposed to toluene at levels of 100 to 1000 ppm for 2 weeks to more than 5 years were reported to be negative (Greenburg et al., 1942); results were not published, but it was noted that the examinations in each case consisted of a "history of ocular complaints, visual acuity, fundus, pupil and slit lamp investigation of the media of the eye."

Raitta and coworkers (1976) found lens changes in a group of 92 car painters who were exposed to a mixture of organic solvents for 1 to 40 years (mean 15 ± 9 years). Of the organic solvents detected in the breathing zones of the workers, toluene was present in the greatest amounts (30.6 ppm); the mean concentrations of the other solvents present in the air are included in the summary of the Hanninen study (Table 11-3). This study was part of a large investigation performed to evaluate the effects of chronic solvent exposure on the nervous system (Hanninen et al., 1976; Seppalainen et al., 1978) and liver function (Kurppa and Husman, 1982) of the car painters (see Sections 11.1. and 11.3). Among the 92 car painters (mean age 34.9 ± 10.4 years, range 21 to 64 years), 2 had been operated on for a cataract and 46 had ocular changes that consisted mainly of lens opacities and/or nuclear sclerosis. To eliminate the influence of age on the development of the lens changes, the painters were compared with age-matched unexposed railroad engineers; 69 age-matched pairs were generated for comparison. Results showed that, in 27 instances, more lens changes were present in the car painters than in the age-matched engineers, and that in 4 instances, there were more changes in the engineers (Table 11-13).

TABLE 11-13

Frequency of Lens Changes and Distribution by Exposure Time in 69 Age-Matched Pairs of Car Painters and Railway Engineers[a]

Result	Frequency of Lens Changes (no. pairs)	Distribution of Lens Changes by Years of Exposure		
		≤ 10	11 to 20	>21
Car painters had fewer changes than the engineers	4	3	1	0
No noticeable difference between the pairs	38	22	13	3
Car painters had more changes than the engineers	27	6	17	4

[a] Source: Raitta et al., 1976

In the remaining 38 pairs, both the painters and the unexposed engineers had similar lens changes. The lens changes were further found to occur with increased frequency after 10 years of exposure (Table 11-13).

11.7.2. Sensory Thresholds. Gusev (1965) investigated the olfactory threshold for toluene in 30 subjects with a total of 744 observations. The minimum perceptible concentration was found by this Russian investigator to be within 0.40 to 0.85 ppm (1.5 to 3.2 mg/m^3), and the maximum imperceptible concentration within 0.35 to 0.74 ppm (1.3 to 2.8 mg/m^3). In sniff tests with 16 subjects (8 male, 8 females), May (1966) determined the minimum perceptible concentration to be a much higher 37 ppm (140 mg/m^3); toluene was found to be clearly perceptible at 70 ppm. In the latter study, the number of observations used to establish the average values were not stated.

Odor thresholds and sensory responses to inhaled vapors of "toluene concentrate" were more recently determined by Carpenter et al. (1976). "Toluene concentrate" is a hydrocarbon mixture containing 45.89% toluene, 38.69% paraffins, 15.36% naphthenes, and 0.06% benzene. The most probable concentration for odor threshold, determined in two trials with 6 subjects, was 2.5 ppm. Based on sensory thresholds for irritation (eye, nose, throat), dizziness, taste, and olfactory fatigue, 6 of 6 volunteers indicated their willingness to work for 8 hours in a concentration of 480 ppm (corresponding to 220 ppm of toluene). Only 3 subjects thought they could work in an atmosphere containing 930 ppm (corresponding to about 427 ppm toluene).

11.8. EFFECTS ON THE SKIN

Toluene appears to be absorbed less readily through the skin than through the respiratory tract, but percutaneous absorption of liquid toluene may be significant (Section 13.1.). When toluene is applied to the skin, its degreasing action will remove natural lipids, possibly causing dryness, fissures, and contact dermatitis (Gerarde, 1960; Browning, 1965).

Malten et al. (1968) found that exposure of human forearm skin for 1 hour on 6 successive days to toluene (volume and conditions not stated) resulted in injury to the epidermal stratum corneum (horny layer). The skin damage was assayed by measurements of water vapor loss, and daily measurements following the exposures indicated that regeneration took about 4 weeks.

Koilonychia and hapalonychia of the fingernails (conditions in which the nails are, respectively, concave and uncornified (soft)) were observed in 6 of 16 cabinet makers who were dermally exposed to a thinner mixture that contained 30% toluene, 30% xylene, and 40% methyl alcohol (Ancona-Alayon, 1975). These deformities involved primarily the thumb, index, and middle fingernails, and were attributed to the practice of cleaning metal parts on furniture with solvent-soaked rags and unprotected hands. Most of the affected workers had an average exposure of 2 years.

11.9. SUMMARY

Toxicity studies in humans have primarily involved evaluation of individuals exposed to toluene via inhalation in experimental or occupational settings or during episodes of intentional abuse, and the health effect of greatest concern is dysfunction of the CNS.

Single 8-hour experimental (Von Oettingen et al., 1942a, 1942b; Carpenter et al., 1944) and subchronic occupational (Wilson, 1943) exposures to toluene in the range of 200 to 300 ppm have elicited subjective symptoms indicative of CNS toxicity (e.g., fatigue, nausea, muscular weakness, mental confusion, and impaired coordination). These effects were generally dose-dependent and increased in severity with increasing toluene concentration. Acute experimental exposures to toluene have also caused objective increases in reaction time at 200 to 300 ppm (Ogata et al., 1970; Gamberale and Hultengren, 1972) and decreases in perceptual speed at 700 ppm (Gamberale and Hultengren, 1972). Gusev (1965) observed disturbances of EEG activity in several subjects exposed to 0.27 ppm toluene for 6-minute intervals, but this effect does not have any apparent toxicological significance.

Short-term accidental workplace (Lurie, 1949; Browning, 1965; Longley et al., 1967; Reisin et al., 1975) and deliberate (Press and Done, 1967a, 1967b; Wyse, 1973; Lewis and Patterson, 1974; Hayden et al., 1977; Oliver and Watson, 1977; Barnes, 1979; Helliwell and Murphy, 1979) inhalation exposures to excessive levels of toluene (i.e., levels approaching air saturation concentrations of 30,000 ppm) have initially resulted in CNS stimulatory effects such as exhilaration, lightheadedness, dizziness, and delusions. As exposure durations increase, narcotic effects characteristic of CNS depression progressively develop, and, in extreme cases, collapse, loss of consciousness, and death (Winek et al., 1968; Chiba, 1969; Nomiyama and Nomiyama, 1978) have occurred.

Chronic occupational exposure to toluene has been associated with "nervous hyperexcitability" (Parmeggiani and Sassi, 1954) and subjective memory, thinking, and activity disturbances (Munchinger, 1963) in workers exposed, respectively, to concentrations of 200 to 800 ppm and 300 to 430 ppm. No evidence of adverse neurological effects have been reported, however, in other studies of printers exposed to 200 to 400 ppm toluene (Suhr, 1975) or manufacturing workers exposed to 80 to 160 ppm toluene (Capellini and Alessio, 1971), although the negative findings in the former study are equivocal and symptoms of stupor, nervousness, and insomnia were noted in one worker exposed to higher concentrations (210 to 300 ppm) of toluene in the latter study. Exposure to mixtures of vapors from an organic solvent containing predominately low-levels of toluene (approximately 30 ppm) for an average of 15 years has produced a greater incidence of CNS symptoms and impaired performance on tests for intellectual and psychomotor ability and memory in car painters (Hanninen et al., 1976; Seppalainen et al., 1978). Matsushita et al. (1975) reported impaired performance in neurological and muscular function tests in female shoemakers who had been exposed to 15 to 200 toluene for an average duration of over 3 years, but these workers were also exposed to "slight" levels of gasoline. Changes in EEG response to photic stimulation were reported by Rouskova (1975) in workers exposed to >250 ppm toluene and unspecified levels of 1,1,1-trichloroethane for an average of 13.5 years.

Residual effects indicative of cerebellar and cerebral dysfunction have been observed in a number of persons who had abused toluene or solvent mixtures containing toluene over a period of years (Grabski, 1961; Satran and Dodson, 1963; Knox and Nelson, 1966; Kelly, 1975; Boor and Hurtig, 1977; Weisenberger, 1977; Keane, 1978; Sasa et al., 1978; Tarsh, 1979; Malm and Lying-Tunell, 1980). These effects were largely reversible upon cessation of exposure, but prolonged toluene abuse has, on occasion, led to permanent encephalopathy and brain atrophy (Knox and Nelson, 1966; Boor and Hurtig, 1977; Sasa et al., 1978). Reports of polyneuropathies in abusers of glues and solvents have also appeared in the literature, but have in all cases involved mixtures of toluene and other solvents such as n-hexane and methyl ethyl ketone (Matsumura et al., 1972; Takenaka et al., 1972; Goto et al., 1974; Shirabe et al., 1974; Suzuki et al., 1974; Korobkin et al., 1975; Oh and Kim, 1976; Towfighi et al., 1976; Altenkirch et al., 1977).

Early reports of occupational exposures (generally prior to the 1950s) ascribed myelotoxic effects to toluene (Greenburg et al. 1942; Wilson, 1943), but the majority of recent evidence indicates that toluene is not toxic to the blood or bone marrow (Von Oettingen et al., 1942a, 1942b; Parmeggiani and Sassi, 1954; Banfer, 1961; Capellini and Alessio, 1971; Suhr, 1975; Matsushita et al., 1975). When administered orally to leukemia patients, it has been further reported that toluene was nontoxic and had no effect on the leukemic process (Francone and Braier, 1954). Hematological abnormalities have been infrequently reported in sniffers of toluene-based glues and thinners (Christiansson and Karlsson, 1957; Massengale et al., 1963; Sokol and Robinson, 1963; Barman et al., 1964; Press and Done, 1967b). Other investigators have noted increases in prothrombin time (Pacseri and Emszt, 1970), decreases in phagocytic activity of leukocytes (Bansagi, 1968), and increased enzyme concentrations in leukocytes and lymphocytes (Friborska, 1973) of workers who were exposed to toluene. Decreases in serum immunoglobin and complement levels (Lange et al., 1973a; Smolik et al., 1973) and leukocyte agglutinins (Lange et al., 1973b) have been reported in workers exposed simultaneously to benzene, toluene, and xylene.

Liver enlargement was reported in an early study of painters with exposures to 100 to 1100 ppm toluene for 2 weeks to more than 5 years (Greenburg et al., 1942), but this effect was not associated with clinical or laboratory evidence of disease or corroborated in subsequent studies of workers (Parmeggiani and Sassi, 1954; Suhr, 1975). Chronic occupational exposure to toluene has generally not been associated with abnormal liver function (Greenburg et al., 1942; Parmeggiani and Sassi, 1954; Capellini and Alessio, 1971; Suhr, 1975; Kurppa and Husman, 1982), although reductions in serum bilirubin and alkaline phosphatase (Szadkowski et al., 1976) and increases in gamma glutamyl transpeptidase (Trevisan and Chiesura, 1978) have been noted. Intensive exposure to toluene via glue or thinner sniffing appears to have a minimal effect on liver function indices (Christiansson and Karlsson, 1957; Grabski, 1961; Massengale et al., 1963; Sokol and Robinson, 1963; Barman et al., 1964; Boor and Hurtig, 1977; Press and Done, 1967a, 1967b).

Exposure to mean concentrations of 100 to 1100 ppm toluene for 2 weeks to 5 years (Greenburg et al., 1942) or 60 to 100 ppm toluene for over 3 years (Matsushita et al., 1975) did not result in abnormal urinalysis findings in airplane painters or female shoemakers, respectively. Glomerular filtration rate was reduced in rotogravure workers with uncharacterized toluene exposures (Askergren et al., 1981), but clinical case reports have described proteinuria and hematuria (Lurie, 1949; O'Brien et al., 1971) and myoglobenuria and renal failure (Reisin et al., 1975) in workers who were accidentally overexposed to toluene. Pyria, hematuria, and proteinuria have been the most frequently observed signs of renal dysfunction associated with the deliberate inhalation of toluene-based glues, but these effects have not been universally observed in glue sniffers (Christiansson and Karlsson, 1957; Massengale et al., 1963; Sokol and Robinson, 1963; Barman et al., 1964; Press and Done, 1967a, 1967b). Several reports have recently appeared that associate deliberate inhalation of toluene with metabolic acidosis (Taher et al., 1974; Fischman and Oster, 1979a; Koeger et al., 1980; Bennett and Forman, 1980; Moss et al., 1980).

Acute experimental exposure to toluene within the range of 50 to 800 ppm have not caused any definite effects on heart rate or blood pressure (Von Oettingen et al., 1942a, 1942b; Ogata et al., 1970; Astrand et al., 1972; Gamberale and Hultengren, 1972). Toluene has been implicated in a small number of sudden deaths due to solvent sniffing which appear to result from cardiac arrhythmias (Bass, 1970; Alha et al., 1973), but trichloroethane and fluorinated aerosol propellants have most frequently been associated with these deaths.

Subjective complaints of dysmenorrhea have been reported by a significant number of female shoemakers exposed to 60 to 100 ppm toluene and concomitantly to 20 to 50 ppm gasoline in a "few" working places for an average duration of 3 years and 4 months (Matsushita et al., 1975). Disturbances of menstruation have also been reported in women exposed concurrently to toluene, benzene, and xylene in the workplace (Michon, 1965), and in women exposed occupationally to toluene and other unspecified solvents (Syrovadko, 1977), but a specific effect of toluene could not be determined from the available data. Information on the possible reproductive effects of toluene in males is not available.

Minimum perceptible concentrations of toluene have been determined to be 0.40 to 0.85 ppm (Gusev, 1965) and 37 ppm (May, 1966), but the reasons for this discrepancy are not apparent. Toluene has been reported to cause transitory eye and respiratory tract irritation as a result of 8-hour exposures in the range of 200 to 800 ppm (Carpenter et al., 1944; Parmeggiani and Sassi, 1954; Capellini and Alessio, 1971), but no complaints of respiratory tract discomfort were recorded in volunteers or workers exposed to levels as high as 800 to 1500 ppm for 8-hour periods in other studies (Von Oettingen et al., 1942a,b; Wilson, 1943). No complaints of respiratory tract or eye irritation were recorded in men accidentally exposed to 10,000 to 30,000 ppm toluene for brief durations (Longley et al., 1967).

Transient epithelial injury to the eyes that healed within 48 hours was observed in workers who were accidently splashed with toluene (McLaughlin, 1946; Grant, 1962). Opthalmologic examinations of spray painters who were exposed to 100 to 1000 ppm toluene for 2 weeks to more than 5 years were normal (Greenburg et al., 1942), but Raitta et al. (1976) found lens changes in a group of car painters exposed concurrently to approximately 30 ppm toluene and much lower concentrations of other solvents for an average of 15 years. The little information that is available on the dermal toxicity of toluene indicates that moderate contact may cause skin damage due to its degreasing action (Gerarde, 1960; Browning, 1965; Malten et al., 1968).

11.10 REFERENCES

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12. ANIMAL TOXICOLOGY

12.1. SPECIES SENSITIVITY

Information on the toxic effects of chronic exposure to low levels of toluene may be more relevant to greater numbers of people than information on acute toxicity (see Sections 10.3 and 10.4). However, for those rare exposures to high levels of toluene, data obtained from acute toxicity studies are valuable. In the sections to follow, consideration will be given to acute as well as chronic studies.

Inhalation has been the principal route of exposure in humans; therefore, animal studies have centered on intoxication by this route. In all species studied, the progressive symptoms typically found after increasingly higher doses are irritation of the mucous membranes, incoordination, mydriasis, narcosis, tremors, prostration, anesthesia, and death. Cats appeared to be more resistant than dogs and rabbits; rats and mice are less resistant than dogs or rabbits (Tables 12-1 and 12-2).

12.1.1. Acute Exposure to Toluene

12.1.1.1. ACUTE INHALATION -- Carpenter et al. (1976a) reported 100% mortality in rats that were exposed for 4 hours to 12,000 ppm of "toluene concentrate" (a mixture of paraffins, naphthenes, and aromatics that contained 45.9% toluene and 0.06% benzene). Tremors were seen in 5 minutes and prostration in 15 minutes. At 6300 ppm, inhalation produced head tremors in 1 hour and prostration in 2 hours, while only slight loss of coordination was seen after 4 hours' exposure to 3300 ppm. A calculated LC_{50} of 8800 ppm for a 4-hour period of inhalation of "toluene concentrate" was reported. Inhalation of a thinner containing less toluene (≈33%) and only 0.01% benzene elicited less toxic symptoms at a similar range of doses in rats in a companion study by the same laboratory (Carpenter et al., 1976b).

In a study by Smyth et al. (1969a), inhalation of 4000 ppm technical grade toluene for 4 hours produced 1 death in 6 rats. In an early study, Batchelor (1927) noted that inhalation of 1600 ppm of toluene for 18 to 20 hours daily produced initial effects of instability and incoordination, conjunctivitis, and lacrimation, and subsequently narcosis and mild twitching. A drop in body temperature followed by death, occurred after 3 days of exposure. At necropsy, a severe cloudy swelling of the kidneys was found. In this study, no effects on

TABLE 12-1

Acute Effects of Toluene

Route	Species	Dose	Effect	Reference
inhalation	rats	24,400 ppm for 1.5 h	60% mortality	Cameron et al., 1938
inhalation	rats	12,200 ppm for 6.5 h	50% mortality	Cameron et al., 1938
inhalation	rats	13,269 ppm	Lethal dose	Faustov, 1958
inhalation	rats	4,000 ppm for 4 h	1/6 dead	Smyth et al., 1969a
inhalation	rats	12,000 ppm for 4 h ("toluene concentrate")[a]	Lethal dose	Carpenter et al., 1976b
inhalation	rats	8,800 ppm for 4 h ("toluene concentrate")[a]	LC_{50}	Carpenter et al., 1976b
inhalation	rats	6,300 ppm for 4 h ("toluene concentrate")[a]	Head tremors in 1 h Prostration in 2 h, normal 3 h after exposure	Carpenter et al., 1976b
inhalation	rats	3,300 ppm for 4 h ("toluene concentrate")[a]	Slight loss of coordination	Carpenter et al., 1976b
inhalation	rats	1,700 ppm for 4 h ("toluene concentrate")[a]	No-effect-level	Carpenter et al., 1976b
inhalation	mice	24,400 ppm for 1.5 h	10% mortality	Cameron et al., 1938
inhalation	mice	12,200 ppm for 6.5 h	100% mortality	Cameron et al., 1938
inhalation	Swiss mice	5,320 ppm for 7 h (less than 0.01% benzene present)	LC_{50}	Svirbely et al., 1943

TABLE 12-1 (cont.)

Route	Species	Dose	Effect	Reference
inhalation	mice	6,942 ppm for 6 h (99.5% purity)	LC_{50}	Bonnet et al., 1979
inhalation	mice	6,634 ppm	LC_{50}	Faustov, 1958
inhalation	mice	9,288 ppm	Lethal dose	Faustov, 1958
inhalation	mice	8,600 ppm or 15,000 ppm ("toluene concentrate")[a]	50% reduction respiratory rate	Carpenter et al., 1976b
inhalation	mice	5,000 ppm ("toluene concentrate")[a]	No-effect-level for respiratory rate	Carpenter et al., 1976b
inhalation	cats	7,800 ppm for 6 h ("toluene concentrate")[a]	Progressive signs: slight loss of coordination, mydriasis, slight hypersensitivity to light within 20 min, prostration within 80 min, anesthesia within 2 hours. One death during 14 d observation period	Carpenter et al., 1976b
inhalation	guinea pigs	4,000 ppm for 4 h	2/3 dead within a few days	Smyth and Smyth, 1928
inhalation	rabbits	5,500 ppm	Lethal within 40 min	Carpenter et al., 1944
inhalation	dogs	850 ppm for 1 h	Increased respiration rate, decreased respiration volume	Von Oettingen et al., 1942b
inhalation	dogs	760 ppm "toluene concentrate" 6 h/d x 2 d rested for 4 d, exposed again for 3 d	Weight loss of 1.1 kg in 1 of 2 dogs, otherwise normal	Carpenter et al., 1976b

TABLE 12-1 (cont.)

Route	Species	Dose	Effect	Reference
inhalation	dogs	1,500 ppm "toluene concentrate" 6 h/d x 3 d	Slight lacrimation and head tremors (2 dogs exposed)	Carpenter et al., 1976b
oral	rats	7.53 g/kg (6.73 to 8.43)	LD_{50}	Smyth et al., 1969a
oral	Wistar rats adult	7.0 g/kg	LD_{50}	Wolf et al., 1956
oral	Sprague-Dawley rats (150 to 200 g)	5.58 g/kg (5.3 to 5.9 g/kg)	LD_{50}	Withey and Hall, 1975
oral	rats 14 d old, both sexes young adults older adults	 3.0 mℓ/kg (2.6 g/kg) 6.4 mℓ/kg (5.5 g/kg) 7.4 mℓ/kg (6.4 g/kg)	 LD_{50} LD_{50} LD_{50}	Kimura et al., 1971
i.p.	rats and mice	2.0 mℓ/kg (1.7 g/kg)	Lethal dose	Cameron et al., 1938
i.p.	rats	0.75 mℓ/kg (0.7 g/kg)	Apathy	Batchelor, 1927
i.p.	rats	1.75 to 2.0 mℓ/kg (1.5 g/kg to 1.7 g/kg)	Death from respiratory failure	Batchelor, 1927
i.p.	rats (both sexes)	800 mg/kg at 26°C 530 mg/kg at 8°C 255 mg/kg at 36°C	Approximate lethal dose	Keplinger et al., 1959
i.p.	mice (male)	1.15 g/kg in olive oil (1.04 to 1.31 g/kg) (graded doses between 0.79 and 1.65 g/kg)	LD_{50} Observed for 24 h Cause of death: respiratory failure	Koga and Ohmiya, 1978
i.p.	mice (female)	1.64 g/kg	LD_{50}	Ikeda and Ohtsuji, 1971

TABLE 12-1 (cont.)

Route	Species	Dose	Effect	Reference
i.p.	mice	4 g/kg	Lethal dose	Tsuzi, 1956
i.p.	guinea pigs	2.0 mℓ pure solvent (1.7 g)	6/10 dead after 2 h All dead after 6 h	Wahlberg, 1976
s.c.	rats and mice	5 to 10 mℓ/kg (4.3 to 8.2 g/kg)	Lethal dose	Cameron et al., 1938
i.v.	rabbits	0.15 mℓ/kg (.13 g/kg) 0.20 mℓ/kg (.17 g/kg)	13% mortality 100% mortality	Braier, 1973
dermal (single application)	rabbits	14.1 mℓ/kg	LD_{50}	Smyth et al., 1969a
dermal	rabbits	uncovered application to abdomen	Slight irritation	Smyth et al., 1969a
dermal	rabbits	10 to 20 applications of undiluted toluene to rabbit ear and bandaged to shaved abdomen	Perceptible erythema, thin layer of devitalized tissue which exfoliated No effect on gross appearance, behavior, or weight	Wolf et al., 1956
dermal	guinea pigs	1 mℓ for 16 h	Karyopyknosis, karyolysis, perinuclear edema, spongiosis, junctional separation, cellular infiltration in dermis, no liver or kidney damage	Kronevi et al., 1979

TABLE 12-1 (cont.)

Route	Species	Dose	Effect	Reference
dermal	guinea pigs	2.0 mℓ, covered	Completely absorbed by 5th to 7th d No mortality up to 4 wk Weight less than controls for wk 1 to 3, no difference at wk 4	Wahlberg, 1976
corneal	rabbits	0.005 mℓ	Moderately severe injury	Smyth et al., 1969a
corneal	rabbits	0.005 mℓ	Moderately severe injury	Carpenter and Smyth, 1946
corneal	rabbits	2 drops	Perceptible irritation of conjunctival membranes No corneal injury	Wolf et al., 1956

h = hour; min = minute; d = day; wk = week; i.p. = intraperitoneal; s.c. = subcutaneous;
i.v. = intravenous; n = number; ns = not specified

[a] "toluene concentrate" is a mixture of paraffins, naphthenes and aromatics that contained 45.9% toluene and 0.06% benzene.

TABLE 12-2

Subchronic Effects of Toluene

Species	Route	Dose	Effect	Reference
Rat	Inhalation	1600 ppm 18 to 20 h/d	Initial effect of instability and incoordination, conjunctivitis, lacrimation, and sniffles; then narcosis	Batchelor, 1927
Rat	Inhalation	1600 ppm 18 to 20 h/d x 3 d	Mild twitching; drop in body temperature; death. Histology: severe cloudy swelling of kidneys, no effect on liver, heart, or testes	Batchelor, 1927
Rat	Inhalation	1250 ppm 18 to 20 h/d	Slight instability and incoordination; mucous membrane irritation	Batchelor, 1927
Rat	Inhalation	620 ppm or 1100 ppm 18 to 20 h/d	No-effect-level for symptoms; hyperplasia of bone marrow	Batchelor, 1927
Rat	Inhalation	1000 ppm solvent mixture (50 to 60% benzene, 30 to 35% toluene, 4% xylene) 7 h/d x 5 d x 28 wk	No effect on body weight; lymphopenia followed by leucocytosis and lymphocytosis; transient changes in blood picture before or after each daily exposure; splenic hemosiderosis greater than that found after inhalation of benzene only: slight to moderate reduction 2-1/2 wk after exposure. Narrowing of peri-follicular collars of cells in spleen, no fat in livers and kidney; iron-negative pigment in kidneys of a few animals.	Svirbely et al., 1944

TABLE 12-2 (cont.)

Species	Route	Dose	Effect	Reference
Rat	Inhalation	240, 480, or 980 ppm "toluene concentrate"[a] 6 h/d x 5 d/wk x 65 d	No effect on red blood cell count, white blood cell count, hematocrit, hemoglobin, total and differential white count, blood urea nitrogen, SGOT, SGPT, alkaline phosphatase, or body weight.	Carpenter et al., 1976b
Rat	Inhalation	3184 ppm 4 h/d x 30 d	Increased levels of SGOT, SGPT, β-lipoproteins Decreased levels of glutathione, catalase, peroxidase, total cholesterol	Khinkova, 1974
Rat	Inhalation	200 ppm or 600 ppm	No narcosis; body weight change in WBC count, RBC count,or hemoglobin during weekly sampling; increase in percentage of segmented cells; histological changes: slight pulmonary irritation; few casts in straight collecting tubules in rats at 600 ppm; no change in liver, spleen, heart, or bone marrow	Von Oettingen et al., 1942b

TABLE 12-2 (cont.)

Species	Route	Dose	Effect	Reference
Rat	Inhalation	2500 ppm or 5000 ppm 7 h/d x 5 d x 5 wk	Transient decrease in body weight; hyperactivity, marked incoordination, recovery after cessation of exposure; mortality in 5000 ppm group 18/25; increased bleeding time; blood picture: total leucocytes reduced after each exposure; pulmonary lesions occurred earlier than in group exposed to 200 or 600 ppm; casts in renal tubules in all rats within 2 wk of exposure; rest of histology same as 200 and 600 ppm	von Oettingen et al., 1942b
		5 d/wk x 15 wk	cytochrome P-450, ethoxycoumarin O-deethylase increased; UDP glucuronslytransferase increased only at end of exposure	
Rats	Inhalation	7 consecutive cycles daily, 5 d/wk x 8 wk: each cycle, 10 min to 12,000 ppm followed by 20 min toluene-free internal	Depression of body weight gain; increased SGOT, LDH levels; no effect on BUN levels Depression of kidney, brain, and lung weights. Histology: no effect on brain, lung, liver heart, or kidney, no sign of lipid vacuolation in liver	Bruckner and Peterson, 1981a
CFY rats (both sexes)	Inhalation	265 ppm 6 h/d x 5 d/wk z 1, 3 or 6 mo	Bromsulphthalein retention decreased; Cytochrome P-450 increased independent of period of exposure; SGOT and SGPT activity unaffected	Ungvary et al., 1980

TABLE 12-2 (cont.)

Species	Route	Dose	Effect	Reference
CFY rats (both sexes)	Inhalation	929 ppm 8 h/d x 5 d/wk x 1 wk, 6 wk, 6 mo	Cytochrome P-450 increased independent of exposure period; no effect on SGOT or SGPT; aniline hydroxylase and aminopyrine N-demethylase activity increased; cytochrome b_5 concentrations increased. Histological effects: dilation of cisternae of rough endoplasmic reticulum; increase of autophagous bodies which was dose and time dependent; retarded growth of females but not males; glycogen content decreased	
CFY rats (males)	Inhalation	398, 796, 1592 ppm 8 h/d x 5 d/wk x 4 wk	Cytochrome P-450 increased with dose	
Rat, guinea pig, dog, monkey	Inhalation	107 ppm continuously for 90 d, or 1085 ppm 8 h/d, 5 d/wk x 6 wk	No effect on leukocytes, hemoglobin, or hematocrit; no effect on liver, kidney, lungs, spleen or heart; no effect on brain or spinal cord of dogs and monkeys	Jenkins et al., 1970

TABLE 12-2 (cont.)

Species	Route	Dose	Effect	Reference
Mice	Inhalation	7 consecutive cycles daily, 5 d/wk x 8 wk: each cycle, 10 min. to 12,000 ppm followed by 20 min. toluene-free interval	Depression of body weight gain; no effect on LDH; decreased BUN levels; SGOT levels increased (not significantly) depression of kidney, brain and lung weights; Histology: no effect on brain, lung, liver, heart, or kidneys; no sign of lipid vaculoation in liver.	Bruckner and Peterson, 1981a
Mice	Inhalation	4000 ppm 99.9% pure toluene for 3 h	No effect on LDH activity significant increase of SGOT 24 h post exposure only	Bruckner and Peterson, 1981b
Mice	Inhalation	4000 ppm 99.9% pure toluene for 3 h/d x 1, 3, or 5 d	SGOT levels increased after 1 and 3 days of treatment; no effect 24 h after 5 d	Bruckner and Peterson, 1981b
Mice	Inhalation	4000 ppm 99.9% pure toluene for 3 h/d x 5 d wk x 8 wk	Depression of body weight gain during first 7 wk; increased liver-to-body weight ratio after 4 wk exposure, no effect at 1, 2, or 8 wk; no increase in kidney, brain, or lung weights; SGOT activity increased after 4 wk of exposure and 2 wk post-exposure, but not after 2 wk or 8 wk of exposure; no change in BUN. Histology: no effect on heart, lung, kidney, brain and liver	Bruckner and Peterson, 1981b

TABLE 12-2 (cont.)

Species	Route	Dose	Effect	Reference
Mice	Inhalation	1, 10, 100 or 1000 ppm 6 h/d x 20 d	No effect on body weight; 1 and 10 ppm caused increase of RBC count on 10th day, recovery on day 20; 100 ppm, 1000 ppm - decrease of RBC count; all doses - increase (40 to 70%) of WBC count on day 10, recovery for all doses except 1000 ppm; 10 ppm to 1000 ppm - decrease of thrombocytes; histology: 100 ppm - slight decrease in density of bone marrow cells and in megakaryocytes and red cell elements; 1000 ppm - slight hypoplasia of red cell elements, slight to moderate disturbance in maturity of neutrophils and thrombocytes, moderate increase of reticulocytes, no change in brain, lung, liver, spleen, or kidney.	Horiguchi and Inoue, 1977
Guinea pig	Inhalation	1250 ppm 4 h/d x 6 d/wk (18 exposures)	Prostration, marked liver and renal degeneration, marked pulmonary inflammation	Smyth and Smyth, 1928
		1000 ppm 4 h/d x 6 d/wk (35 exposures)	No symptoms; slight toxic degeneration in liver and kidney	

TABLE 12-2 (cont.)

Species	Route	Dose	Effect	Reference
Dogs 2 experimental, 1 control	Inhalation	2000 ppm 8 h/d x 6 d/wk x 4 mo, and then 2660 ppm 8 h/d, 6 d/wk x 2 mo	Death on days 179 and 180; slight nasal and ocular irritation; motor incoordination and paralysis of extremities during terminal phase; congestion in lungs, hemorrhagic liver, reduced lymphoid follicles and hemosiderosis in spleen; hyperemic renal glomeruli; albumin in urine	Fabre et al., 1955
Dogs	Inhalation	200, 400, 600 ppm 3 8 h exposures for 1 wk, then 5 x 7 h for 1 wk and finally 850 ppm for 1 hr	No effect on circulation, spinal pressure; increase of respiratory rate, small increase of minute volume, decrease of respiratory volume	von Oettingen et al., 1942b
Dogs	Inhalation	400 ppm; 7 h/d x 5 d	Moderate temporary lymphocytosis	von Oettingen et al., 1942b
Rats	Oral	118 mg/kg/d, 354 mg/kg/d, 590 mg/kg/d x 138 d	No effect on body and organ weights, adrenals, pancreas, femoral bone marrow, lungs, heart, liver, kidney, spleen, testes, bone marrow, BUN, blood counts	Wolf et al., 1956

TABLE 12-2 (cont.)

Species	Route	Dose	Effect	Reference
Rats	Subcutaneous	1 cc/kg x 21 d	Slight induration at injection site; 5 to 14% loss of body weight; transient slight drop in RBC and WBC counts; hyperplasia of bone marrow; moderate hyperplasia of malpighian corpuscle in spleen; marked pigmentation of spleen; focal necrosis in liver; slight cloudy swelling in kidney; no effect on heart, testes, or lungs	Batchelor, 1927
Guinea pig	Subcutaneous	0.25 cc/d x 30 to 70 d	Local necrosis at injection site; survival period: 30 to 70 days; polypnea and convulsions during last days of survival; hemorrhagic, hyperemic, and sometimes degenerative changes in lungs, kidneys, secondary adrenals, liver, and spleen	Sessa, 1948

TABLE 12-2 (cont.)

Species	Route	Dose	Effect	Reference
Rabbit	Subcutaneous	1 cc/kg x 6 d	Transient slight granulopenia followed by granulocytosis; no change in bone marrow	Braier, 1973
		4 cc/kg	More marked effect on granulocytes; all rabbits dead by end of second day; no effect on bone marrow	

h = hour; d = day; wk = week; SGOT = serum glutamic oxalacetic transaminase; SGPT = serum glutamic pyruvic transaminase; WBC = white blood cell; RBC = red blood cell; UDP = uridine 5'-phosphate; BUN = blood urea nitrogen; mo = month.

the liver, heart, or testes were observed, although hyperplasia of the bone marrow was noted, suggesting possible contamination of the solvent with benzene.

Cameron et al. (1938), found that 24,400 ppm of toluene produced a mortality of 60% and 10% in rats and mice, respectively, after 1.5 hours of exposure. In another group of rats and mice exposed to one-half the above concentration for a longer period, 6.5 hours, the mortality was 50% and 100%, respectively. These two species are probably equally sensitive. Other studies of mice include that of Svirbely et al. (1943), in which the 7-hour LC_{50} in Swiss mice was determined to be 5320 ppm, and that of Bonnet et al. (1979), in which a 6-hour LC_{50} of 6942 ppm for mice was noted.

In the study of Carpenter et al. (1976b), 4 of 4 cats initially survived exposure to inhalation of 7800 ppm "toluene concentrate" for 6 hours. During exposure, the cats showed progressive signs of toxicity, including slight loss of coordination, mydriasis, slight hypersensitivity to light within 20 minutes, prostration within 80 minutes, and light anesthesia within 2 hours. Only 1 cat died during the 14-day observation period.

Inhalation of 4000 ppm toluene (purified by distillation) for 4 hours daily was lethal within a few days to 2 of 3 guinea pigs. The third animal was severely prostrated. Under the same regimen, animals exposed to less than one-third of this concentration (1250 ppm) for 6 days a week survived 3 weeks of exposure, although they were severely affected. At 1000 ppm, guinea pigs were not affected even after 35 exposures, although there were slight toxic degenerative changes in the liver and kidney (Smyth and Smyth, 1928).

Carpenter et al. (1944) reported that inhalation of a concentration of about 55,000 ppm was lethal to 6 rabbits in about 40 minutes (range of 24 to 62 minutes).

Von Oettingen et al. (1942b) observed that inhalation of 850 ppm toluene containing 0.01% benzene for 1 hour by 6 dogs produced an increase of respiratory rate and a decrease of respiratory volume. Exposure to 1500 ppm of "toluene concentrate" for 6 hours daily for 3 days produced only slight lacrimation and head tremors in dogs. Reduction of the concentration of "toluene concentrate" to 1000 ppm did not alleviate the head tremors (Carpenter et al., 1976b).

Bruckner and Peterson (1981a,b) found an age-dependent sensitivity in rats and mice. Mice, 4 weeks of age, were found to be more susceptible to exposure of 2600 ppm toluene vapor for 3 hours than 8 or 12 week old animals.

12.1.1.2. ACUTE ORAL TOXICITY -- An LD_{50} of 7.53 g/kg and 7.0 g/kg body weight for a single oral dose in rats has been reported by Smyth et al. (1969a) and Wolf et al. (1956), respectively. Withey and Hall (1975) found 5.58 g/kg to be the LD_{50} in male Sprague-Dawley rats. Immature 14-day-old Sprague-Dawley rats were more sensitive than young or mature adult male rats of the same strain to the acute effects of toluene (analytical grade) in the studies of Kimura et al. (1971). These investigators determined an oral LD_{50} of 2.6 g/kg body weight, 5.5 g/kg body weight, and 6.4 g/kg body weight for each group, respectively. This age-dependent sensitivity was also noted by inhalation exposure (see Section 12.1.1.1.). Cameron et al. (1938), however, reported that very young rats were more resistant to toluene than adult animals of the Wistar strain. Thirty-three percent of a group of 12 nine day old rats survived 5.25 hours of exposure to air saturated with toluene, but 100% mortality was observed in a group of adult rats exposed for the same duration.

Based on the results of their studies on the oral toxicity of toluene in animals of different age groups, Kimura et al. (1971) suggested a maximum permissible limit of 0.002 g/kg bw for a single oral dose. This was obtained by dividing the dose that elicited the first observable gross signs of CNS toxicity by 1000.

12.1.1.3. ACUTE EFFECTS FROM INTRAPERITONEAL INJECTION -- Mortality is produced by a single intraperitoneal injection of toluene in the range of 0.8 to 1.7 g/kg in rats and mice. Koga and Ohmiya (1978) determined an LD_{50} of 1.15 g/kg body weight for male mice from administration of toluene graduated between 0.79 and 1.65 g/kg and diluted in olive oil. Respiratory failure was the main cause of death in these animals. An LD_{50} of 1.64 g/kg was reported for female mice by Ikeda and Ohtsuji (1971). The reason for the disparity in the above data (e.g., interlaboratory differences or sexual differences in sensitivity) has not been ascertained. In rats an i.p. injection of 0.65 g/kg toluene produced apathy, while 1.5 to 1.7 g/kg produced death from respiratory failure (Batchelor, 1927); 1.7 g/kg was a lethal dose in rats, mice (Cameron et al., 1938), and guinea pigs (Wahlberg, 1976).

Savolainen (1978) observed that the concentration of radioactivity in the CNS was highest in the cerebrum after an intraperitoneal injection of methyl ^{14}C-toluene. Label was undetectable in the CNS by 24 hours post-injection, which is consistent with the time course of clinical signs of acute toluene intoxication.

A temperature-dependent sensitivity to toluene was observed in adult rats of both sexes by Keplinger et al. (1959). The lethal dose at 26°C was 800 mg/kg, while at 8°C and 36°C, lethal doses were 530 mg/kg and 225 mg/kg, respectively. The toxicity of toluene is greater in hot and cold environments. It is unknown whether increased susceptibility to toluene is caused by the stress of altered environmental temperature, or by altered physiological processes (e.g., absorption, diffusion, distribution, or metabolic rate) .

12.1.1.4. ACUTE EFFECTS FROM SUBCUTANEOUS INJECTION -- Subcutaneous injection of 1.1 to 1.25 g/kg and 4.3 to 8.7 g/kg toluene have been reported to produce mortality in rats and mice, respectively (Batchelor, 1927; Cameron et al., 1938). Braier (1973) reported that 4 cc toluene/kg injected into rabbits produced marked transient granulopenia within 24 hours and marked granulocytosis and ensuing death in all animals by the end of the second day. A slight area of induration was seen at the injection site.

12.1.1.5. ACUTE EFFECTS FROM INTRAVENOUS INJECTION -- Intravenous injection of 0.2 cc toluene/kg produced 100% mortality in rabbits (Braier, 1973).

12.1.1.6. ACUTE AND SUBACUTE EFFECTS OF PERCUTANEOUS APPLICATION -- Repeated application of undiluted toluene to the rabbit ear or shaved skin of the abdomen produced slight to moderate irritation (Wolf et al., 1956; Smyth et al., 1969a) and increased local capillary permeability (Delaunay et al., 1950). Continuous cutaneous contact in the guinea pig resulted in slowed weight gain, karyopyknosis, karyolysis, spongiosis, and cellular infiltration in the dermis within 16 hours (Kronevi et al., 1979; Wahlberg, 1976). Application to the abdominal skin of the rat produced hemoglobinuria (Schutz, 1960). Slight irritation of conjunctival membranes but no corneal injury (Wolf et al., 1956) or moderately severe injury (Carpenter and Smyth, 1946; Smyth et al., 1969a), have been observed following direct application of toluene to the eye.

12.1.2. Subchronic and Chronic Exposure to Toluene. Subchronic or chronic studies of toluene have not indicated, with the exception of the high exposure level study of Fabre et al. (1955), evidence of major toxic effects.

Fabre et al. (1955) exposed 2 dogs for 8 hours daily, 6 days a week, to 2000 ppm pure toluene via inhalation for 4 months, and subsequently to 2660 ppm for 2 months. Slight nasal and ocular irritation occurred at the lower concentration, and motor incoordination that preceded paralysis of the extremities occurred in the terminal phase. Death occurred on days 179 and 180. There was no effect on gain in body weight, on the bone marrow, or on the adrenal, thyroid, or pituitary glands. Congestion in the lungs, hemorrhage in the liver, a decrease of lymphoid follicles, and hemosiderosis in the spleen were observed. Glomeruli of the kidney were hyperemic, and albumin was found in the urine.

Svirbely et al., (1944) found that repeated inhalation of 1000 ppm of a solvent mixture containing 30 to 35% toluene, 50 to 60% benzene, and a small amount of xylene for 28 weeks (7 hours/day, 5 days/week) had no effect on body weight in rats or dogs. There was no significant increase of liver volume, and fat was not found in the liver or kidneys; however, narrowing of perifollicular collars was observed in the spleen (Table 12-2). Splenic hemosiderosis was greater than that found after exposure to benzene (Svirbely et al., 1944).

Continuous exposure to 107 ppm toluene for 90 days or repeated exposure to 1085 ppm toluene for 6 weeks (8 hours/day, 5 days/week) did not adversely affect the liver, kidney, lungs, spleen, or heart in 30 rats, 30 guinea pigs, 4 dogs, or 6 monkeys. In addition, treatment-related effects were not seen in the brain or the spinal cord of dogs or monkeys. No significant change was observed in hematologic parameters (hemoglobin, hematocrit, or leucocyte count). All animals with the exception of 2 of 30 treated rats survived exposure, and all gained body weight with the exception of the monkeys (Jenkins et al., 1970).

Repeated inhalation of 240, 480, or 980 ppm of "toluene concentrate" for 13 weeks (6 hours/day, 5 days/week) produced no treatment-related organ damage in rats or dogs. Serum alkaline phosphatase (SAP), serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), and blood urea nitrogen (BUN) activities were normal. Prior treatment with toluene did not render the animals either more susceptible or more resistant to a subsequent challenge dose of 12,000 ppm (Carpenter et al., 1976b).

The results of an unreviewed subchronic inhalation study with rats that was performed by Bio/dynamics for the American Petroleum Institute (1980) are available. Groups of 15 Sprague-Dawley rats of each sex were exposed 6 hours per day, 5 days per week for 26 weeks to cumulative concentrations of 0, 100, and 1481 ppm toluene. Initially, the high dose group was exposed to 2000 ppm, but the dose was lowered to 1500 ppm after 7 exposures because CNS depression was apparent. A battery of blood and clinical chemistry tests (BUN, SGPT, SAP, glucose), urinalysis, and neurohistological examination of tissue was performed. The only treatment-related sign was increased incidence of dry rales and staining of the ano-genital fur in the high level treatment group. Significant changes in the blood and urine were not found with the exception of a dose-related decrease in blood glucose levels and a dose-related increase in SGPT levels in female rats. Body weights were significantly higher in the high-dose male rats than in the control rats, but this was not considered a toxic effect. Treatment-related neurohistopathological changes were not found.

Inhalation exposure to 1000 ppm toluene for 6 hours a day, 5 days a week for 6 months had no treatment-related effects on male OFA rats (Gradski et al., 1981). Twenty-four rats/treatment and control group were examined, and body weight gain, hematologic parameters (BBC and WBC counts, hemoglobin, mean corpuscular volume, hematocrit, sedimentation rate), and tissue histology (lungs, liver, spleen, kidney, genitals, and other unspecified "principal" organs) were assessed.

In a chronic inhalation study conducted by Industrial Bio-Test Laboratories, Inc. for the Chemical Industry Institute for Toxicology (CIIT), groups of 120 Fischer 344 rats of each sex were exposed to 30, 100, or 300 ppm of high purity (>99.98%) toluene for 6 hours/day, 5 days/week for 24 months (CIIT, 1980). All animals were weighed at the beginning of the study, weekly for the first 6 months, every other week from 6 to 24 months, and immediately prior to sacrifice. Hematology, blood chemistry, urinalysis, opthamology, and pathology determinations were conducted on randomly selected rats that were sacrificed after 6, 12, or 18 months to determine progression of toxic effects (Table 12-3). All remaining rats were sacrificed for study after 24 months, but histopathological examinations were conducted only on tissues from rats in the high exposure (300 ppm) and control (0 ppm) groups.

Unscheduled deaths occurred in 140 rats (14.6% of 960 animals) over the 2-year course of the study, but mortality in the treated rats reportedly did not differ significantly from controls (CIIT, 1980). The body weights of the treated males were found to be significantly heavier than the control males throughout the study, although a clear dose-response relationship was not apparent (Table 12-4). A similar effect was noted for the females but the effect disappeared during the final 4 weeks of the study. There were, however, no significant differences among the groups in absolute organ weights (brain, liver, heart, kidneys, lungs, and testes or ovaries were weighed). The battery of clinical chemistry tests, hematologic studies and urinalyses (Table 12-3) revealed normal levels in the treated rats except for two hematologic parameters in females. Females exposed to 100 or 300 ppm toluene showed slightly, but significantly, reduced hematocrits, and the mean corpuscular hemoglobin concen-

TABLE 12-3

Number of Rats Per Sex and Treatment Group Examined at Each Interval

Interval	6 Months	12 Months	18 Months	24 Months
Hematology[a]	5	5	20	10
Blood Chemistry[b]	5	5	10	10
Urinalysis[c]	5	5	10	10
Ophthamology[d]	5	5	25[e]	58-66
Pathology[f]	5	5	20	68-76[g]

[a] Hemoglobin concentration (HgB), hematocrit (HcT), total erythrocyte count (RBC), and total and differential leukocyte counts (WBC) were determined; mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were subsequently calculated.

[b] Blood urea nitrogen (BUN), serum glutamic pyruvic transaminase (SGPT) activity, and serum alkaline phosphatase (SAP) were determined.

[c] Appearance, specific gravity, protein or albumin, pH, ketones, glucose, and presence of microscopic particles were determined.

[d] Ophthalmological examinations were conducted on all animals scheduled for interval and final (24-month) sacrifice, except animals also scheduled for blood collection at the final sacrifice.

[e] 20 rats/sex/group were originally scheduled for sacrifice, but an additional 5 rats/group were examined so that they could be used as replacement animals should any of the first group not survive until the final sacrifice date.

[f] Histologic examinations were conducted on 38 tissues taken from the high exposure (300 ppm) and control (0 ppm) rats only. All scheduled sacrifices, as well as rats that were sacrificed in extremis (8-17 rats/sex/group) or died during the course of the study (2-7 rats/sex/group), were examined grossly.

[g] All surviving animals at the end of the 24 months were sacrificed for pathologic examination.

tration was slightly, but significantly, increased in females exposed to 300 ppm (Table 12-5). A variety of inflammatory, degenerative, proliferative, and neoplastic lesions were observed in various tissues (see Section 14.1), but the lesions occurred with equal frequency in all control and treatment groups; CIIT (1980) concluded that no tissue changes can be attributed to toluene inhalation. There were no significant differences among the groups; absolute ophthalmologic examinations did not reveal any toluene-induced changes in the eyes of the rats.

TABLE 12-4

24 Month Chronic Exposure of Fischer 344 Rats Exposed
6 Hours/Day, 5 Days/Week, to Toluene by Inhalation

Group	Number Animals	Mean Body Weight in Grams						Total Weight Change
		Weeks of Exposure						
		0	26	52	78	100	104	
Males								
Control	89	141	340	384	426	430	430	286
30 ppm	89	141	349*	396**	445**	456**	454**	314**
100 ppm	89	141	351**	404**	447**	454**	452**	312**
300 ppm	90	142	341	403**	446**	451**	445	304**
Females								
Control	90	109	203	213	214	260	265	156
30 ppm	90	109	191**	211	246**	272**	273*	164
100 ppm	90	109	194	211	248**	272**	275	166
300 ppm	90	109	195**	211	248**	271**	272	163

[a]Source: CIIT, 1980

*Statistically significant difference from control (P <0.05)

**Statistically significant difference from control (P <0.01)

In the only subchronic oral study, female rats fed up to 590 mg toluene/kg by intubation for periods of up to 6 months did not show ill effects as determined by gross appearance, growth, periodic blood counts, analysis for blood urea nitrogen, final body and organ weights, bone marrow counts, or histopathological examination of adrenals, pancreas, lungs, heart, liver, kidney, spleen, and testis (Wolf et al., 1956).

12.2. EFFECTS ON LIVER, KIDNEY, AND LUNGS

Toxic effects in the kidney, and possibly in the liver and lungs after higher doses, have been reported.

TABLE 12-5

Hematology Measurements
24 Month Chronic Exposure of Fischer 344 Rats Exposed 6 Hours/Day, 5 Days/Week, to Toluene by Inhalation

Group	Number of Animals	WBC (10^3/cu mm)	RBC (10^6/cu mm)	HgB (g/DL)	HcT (%)	MCV (Cu. Mic.)	MCH (μμg)	MCHC (%)
			18 Months of Exposure (Males)					
Control	20	6.03	8.757	16.56	43.10	50.4	18.87	38.04
30 ppm	20	9.96*	8.766	16.61	42.42	49.6	18.90	38.82
100 ppm	20	6.54	8.700	16.47	41.93	49.5	18.91	38.93
300 ppm	20	6.53	8.894	16.80	42.34	48.8**	18.85	39.30**
			24 Months of Exposure (Males)					
Control	10	7.51	9.866	18.91	51.78	51.2	19.24	37.87
30 ppm	10	8.66	8.736	16.58	46.51	52.5	19.05	36.33
100 ppm	10	8.13	9.925	18.47	51.61	50.7	18.67	38.84
300 ppm	10	7.50	9.407	18.33	47.35	50.9	19.44	39.33
			18 Months of Exposure (Females)					
Control	20	4.04	8.022	15.67	41.70	53.0	19.49	37.26
30 ppm	20	4.59	7.956	15.77	41.25	52.8	19.77	37.90*
100 ppm	20	3.91	7.915	15.75	40.83	52.7	19.85*	38.24*
300 ppm	20	4.21	8.010	15.78	41.20	52.4	19.63	37.98
			24 Months of Exposure (Females)					
Control	10	4.93	8.397	16.46	44.99	54.7	19.50	36.10
30 ppm	10	5.40	8.274	15.89	43.06	53.3	19.11	36.42
100 ppm	10	5.74	8.076	15.94	42.47*	53.9	19.68	37.08
300 ppm	10	4.87	8.090	15.86	42.02**	53.1	19.52	37.46*

[a] Source: CIIT, 1980

*Statistically significant difference from control ($P < 0.05$)

**Statistically significant difference from control ($P < 0.01$)

WBC = white blood cell count; RCB = red blood cell count; HgB = hemoglobin concentration; DL = 100 milliliters; HcT = hematocrit; MCV = mean corpuscular volume; Mic. = micron; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.

12.2.1. Liver

Histological damage was not observed after subchronic or chronic inhalation of 1000 ppm of a solvent mixture containing 30 to 35% toluene for 28 weeks, 980 ppm of "toluene concentrate" for 13 weeks, 1085 ppm of toluene for 6 weeks, or 300 ppm of 99.98% pure toluene for 24 months in a variety of species in the studies described in Section 12.1.2. (Svirbely et al., 1944; Carpenter et al., 1976b; Jenkins et al., 1970; CIIT, 1980). Furthermore, no liver damage was detected in female rats after daily oral doses of 590 mg/kg toluene for 6 months (Wolf et al., 1956).

Two preliminary reports (abstracts) from the laboratory of Bruckner and Peterson noted no effect on hepatorenal function. In a regimen designed to mimic solvent "sniffing", male rats and mice were exposed to 12,000 ppm toluene for 7 ten-minute periods (interspersed with 20-minute toluene-free periods), 5 days/week for 8 weeks. No organ pathology was found. Lactic dehydrogenase, SGPT activity, BUN level, and liver triglyceride content were normal (Bruckner and Peterson, 1978). In another study, inhalation of 4000 ppm toluene (3 hours/day, 5 times weekly) for up to 8 weeks failed to reveal toluene induced hepatorenal injury as indicated by a battery of toxicological tests (SGOT activity, BUN level, urinary glucose and protein concentration, and urinary cell count), and histopathological examination of the liver, kidney, and lung (Bruckner and Peterson, 1976).

Intraperitoneal injection of reagent grade toluene (corn oil vehicle) at doses of 150, 300, 600, or 1200 mg/kg had no effect on serum ornithine carbamyl transferase activity in adult male guinea pigs, when assayed 24 hours after injection (Divincenzo and Krasavage, 1974). Histological examination revealed no liver abnormalities or lipid accumulation with the exception of the highest dose, where there was evidence of lipid accumulation.

Two hours after male rats (weighing 150 to 300 g) were administered 239.5 mg/100 g body weight of toluene in mineral oil by gavage, there was no evidence of injury to the microsomal function of the liver. There was no effect on protein synthesis, cell sap RNA, glucose 6-phosphatase, oxidative demethylase, nicotinamide adenine dinucleotide phosphate (NADPH), neotetrazolium reductase, or lipid conjugated diene content of microsomes (Reynolds, 1972). Inhalation of 300 ppm toluene (6 hours/day, 5 days/week) for 15 weeks slightly increased cytochrome P-450 content in the liver, appreciably enhanced ethoxycou marin o-deethylase, and at the end of exposure, increased UDP glucuronyltransferase activity. The content of toluene in perirenal fat tended to decrease during continued exposure, while presence in the brain was detected throughout exposure. The diminuation of toluene content in perirenal fat at the same time that drug metabolizing enzymes increased suggests an adaptation to continued presence of the solvent (Elovaara et al., 1979).

Continuous cutaneous contact with a dose of 1.7 g (2.0 mℓ) toluene, which was completely absorbed within 5 to 7 days, produced no change in liver morphology (Wahlberg, 1976).

Although the studies just cited indicate the absence of toluene-induced hepatic toxicity, there are others that suggest a slight toxic effect. In a study by von Oettingen et al. (1942b), inhalation of 600 to 5000 ppm toluene that contained 0.01% benzene for 5 weeks (7 hours/day, 5 days/week) by rats caused an enlargement of the liver (increase of weight and volume) in a dose-dependent manner 16 hours after the last exposure. Histologically there was a progressive decrease of cytoplasm density in the liver cells as the concentration of toluene increased, but hyperemia was not noted. These observations were not seen in rats sacrificed 2 weeks after the last exposure. Matsumoto et al. (1971) reported an increase in liver weight and liver weight to body weight ratio in rats exposed 9 hours/day, 6 days/week for 43 weeks to 2000 ppm toluene vapor. This was not noted at lower doses (100 ppm or 200 ppm).

In the inhalation study of Fabre et al. (1955), 2 dogs exposed for 4 months (8 hours/day, 6 days/week) to 2000 ppm pure toluene and, subsequently, to 2660 ppm for 2 months, had hemorrhagic livers.

Tahti et al. (1977) observed that inhalation of 1000 ppm toluene, 8 hours/day for 1 week, increased SGOT and SGPT activity and induced metabolic acidosis in rats.

Although early reports from the same laboratory revealed no effect on SGOT activity or BUN level in mice or rats, a recent report by Bruckner and Peterson (1981b) noted an increase in SGOT activity in these species during intermittent exposure to 12,000 ppm toluene (Section 12.3.). Increase in LDH activity was seen in rats and decrease in BUN levels was seen in mice. No histological changes were observed, but an increase of organ weight to body weight ratio was found.

Histological changes in the liver were found when male CFY rats were injected intraperitoneally with 0.43 or 0.87 g/kg body weight of analytical grade toluene for up to 4 weeks (Ungvary et al., 1976). There was a dose-dependent increase in the number of mitochondria per unit of cytoplasmic area in the liver. Total area, nuclear density, and nucleus/cytoplasm ratio increased at the higher dosage. Dose-dependent decreases in nuclear volume were seen after intraperitoneal or subcutaneous injection, with subcutaneous injection being less effective than intraperitoneal injection. The authors suggested that the considerable accumulation of mitochondria was related to increased metabolism by the liver, and that oxidative detoxification of the solvent might involve mitochondrial enzymes as well as hepatic microsomal enzymes. In an earlier paper, Ungvary et al. (1975) found that intraperitoneal or subcutaneous administration of toluene produced degenerative changes, i.e., separation of ribosomes and vacuolar dilation of the rough endoplasmic reticulum. In these studies, the higher concentrations of toluene also decreased glycogen content. Following discontinuation of exposure, the hepatic changes indicating increased load on detoxification processes (increased succinate dehydrogenase (SDH) activity, increase of mitochondria and smooth endoplasmic reticulum, decreased glycogen content) as well as degeneration (dilation of endoplasmic reticulum, accumulation of autophagous vacuoles) rapidly regressed, indicating that the toxic and liver loading effects of toluene are reversible. The regenerative property of the liver after hepatectomy was not significantly affected by exposure to toluene (Hudak et al., 1976).

In a more recent study by Ungvary et al. (1980), male CFY rats were exposed via inhalation to 265 ppm (6 hours daily), 929 ppm or 1592 ppm (8 hours daily) analytical grade toluene, and female rats were exposed to the lowest dose only (5 exposures a week for up to 6 months). Growth was inhibited in males at the higher concentrations, and in the females. No abnormal histological changes were found in the liver, but liver weight was increased by treatment. Signs of adaptive compensation included proliferation of smooth endoplasmic reticulum, increased cytochrome P450 and cytochrome b_5 activity, increased aniline hydroxylase activity, and increased aminopyrine N-demethylase activity. These changes were dose-dependent and reversible, but showed little or no dependence on length of exposure. There was no effect on SGOT or SGPT activity. The authors concluded from their latest studies that subchronic exposure to toluene vapors has no specific hepatotoxic effect. The histological effects of inhalation exposure to toluene were corroborated by the earlier intraperitoneal or subcutaneous studies, with the exception that necrotic areas were not found after inhalation. Whether or not this reflects the different route of exposure or the higher concentration of toluene administered intraperitoneally has not been ascertained.

12.2.2. Kidney. Histological effects of renal toxicity were not seen in subchronic inhalation studies (Table 12-2) mice exposed to 1000 ppm for 20 days (Horiguchi and Inoue, 1977), in rats, guinea pigs, dogs, or monkeys exposed to 1085 ppm for 6 weeks (Jenkins et al., 1970), in rats and mice exposed to 4000 ppm toluene for 8 weeks (Bruckner and Peterson, 1981b), or in chronic inhalation studies in rats exposed to 300 ppm for 24 months (CIIT, 1980). Toluene did not elicit an observable effect in renal histology after daily subchronic oral dosing at a level of 590 mg/kg for 138 days in rats (Wolf et al., 1956).

Pathological renal changes, however, have been observed in some studies. von Oettingen et al. (1942b) found increasing numbers of casts in the collecting tubules of rat kidneys during inhalation of concentrations ranging from 600 ppm to 5000 ppm for 5 weeks (7 hours daily, 5 days/week). A few casts in the kidney were seen after the third week of exposure at 600 ppm and earlier in the higher doses. Appreciable fat in the convoluted tubules and hyaline casts in the collecting tubules were observed in dogs following inhalation exposure to 200 to 600 ppm for approximately 20 daily 8-hour exposures, subsequently to 400 ppm for 7 hours/day, 5 days/week for 1 week, and finally to 850 ppm for 1 hour. Matsumoto et al. (1971) reported that inhalation exposure to toluene at a level of 2000 ppm for 8 hours/day, 6 days/week for 43 weeks produced hyaline droplets in the renal tubules of rats. There was an increase in kidney weight and the ratio of kidney weight to body weight.

Inhalation of 2000 ppm toluene 8 hours/day, 6 days/week for 4 months, followed by exposure to 2600 ppm during the remaining 2 months, produced hyperemic renal glomeruli and albuminuria in dogs (Fabre et al., 1955). In guinea pigs, inhalation of 1000 ppm distilled pure toluene (4 hours/day, 6 days/week for a total of 35 exposures) produced slight toxic degeneration in the kidney (Smyth and Smyth, 1928). Eighteen exposures at a higher levels of 1250 ppm produced more marked degeneration. Degeneration of convoluted tubular epithelium in guinea pigs exposed to toluene by the subcutaneous route was reported in an abstract of a paper by Sessa (1948).

12.2.3. Lungs. Histological lung damage was not seen after inhalation of 1000 ppm toluene for 20 days in mice (Horiguchi and Inoue, 1977), after inhalation of 1085 ppm for 6 weeks in rats, guinea pigs, dogs, or monkeys (Jenkins et al., 1970), after inhalation of 4000 ppm for 8 weeks in rats and mice

(Bruckner and Peterson, 1981b), after inhalation of 300 ppm for 24 months in rats (CIIT, 1980), or after daily ingestion of 590 mg/kg for 138 days in rats (Wolf et al., 1956).

Irritative effects on the respiratory tract, however, have been reported (Browning, 1965; Gerarde, 1959; Fabre et al., 1955; von Oettingen et al., 1942b). Marked pulmonary inflammation was seen in guinea pigs after inhalation of 1250 ppm distilled pure toluene for 4 hours daily, 6 days/week, for 18 exposures (Smyth and Smyth, 1928). Hemorrhagic, hyperemic, and sometimes degenerative pulmonary changes were observed in guinea pigs after a subcutaneous injection of 0.22 g of toluene daily for 30 to 70 days as reported in an abstract (Sessa, 1948). Repeated exposure to concentrations of 200 to 600 ppm toluene produced congestion in the lungs of dogs, and pulmonary lesions were elicited in rats after 1 week of inhalation of 2500 ppm (7 hours/day, 5 days/week) (von Oettingen et al., 1942b). Congestion in the lungs was noted by Fabre et al. (1955) in dogs exposed for 8 hours a day, 6 days a week to 2000 ppm toluene for 4 months, and subsequently to 2660 ppm for 2 months.

12.3. BEHAVIORAL TOXICITY AND CENTRAL NERVOUS SYSTEM EFFECTS

Excessive depression of the CNS has been associated with acute exposure to high levels of toluene. A concentration of 20,000 ppm toluene was lethal to rats after 30 to 50 minutes of exposure, with death attributed to depression of the CNS (Kojima and Kobayashi, 1975, cited in NRC, 1980). Inhalation of 12,000 ppm of "toluene concentrate" containing 0.06% benzene was lethal to rats following tremors that appeared within 5 minutes of exposure, and prostration that occurred within 15 minutes of exposure (Carpenter et al., 1976b).

A dose-related effect on instability, incoordination, and narcosis was found in rats exposed 18 to 20 hours daily to toluene concentrations of 1600 ppm and 1250 ppm (Batchelor, 1927).; no symptoms were seen at 1100 ppm. Carpenter et al. (1976b) reported that rats were unaffected by inhalation exposure to 1700 ppm of "toluene concentrate" for 4 hours, and suffered only slight incoordination at 3300 ppm. Dogs were unaffected by exposure to 760 ppm for 6 hours, but exhibited head tremors at 1500 ppm. After inhalation of 7800 ppm "toluene concentrate" for 6 hours, cats exhibited loss of coordination followed by prostration and, finally, light anesthesia within 2 hours, but no mortality.

Bruckner and Peterson (1981b) observed that the onset of narcosis and the depth of CNS depression was dose-dependent in mice exposed via inhalation to

12,000 ppm, 5200 ppm, or 2600 ppm toluene. Recovery was rapid. After exposure to 12,000 ppm for 20 minutes, mean performance levels scored prior to exposure were restored within approximately one-half hour in 4-week-old rats.

A single intravenous injection of 0.06 g toluene per kg body weight caused generalized rigidity with hyperextension of the back within 10 seconds in an experiment with 1 dog (Baker and Tichy, 1953). Recovery occurred within 12 minutes. When a series of 10 doses of 0.06 g toluene/kg was given intravenously every 3 to 5 days to another dog, rigidity and twitching of the extremities were induced. Recovery occurred in 5 to 10 minutes. At necropsy, cortical and cerebellar atrophy was found. Marked shrinkage and hyperchromaticity of many cortical neurons, patchy myelin pallor and fragmentation, especially in perivascular areas, were found. Multiple fresh petechiae, especially in the white matter, were seen. There was a decrease and degeneration of Purkinje cells in the cerebellum (Baker and Tichy, 1953).

12.3.1. Effect of Solvent-Sniffing Abuse. In the section on CNS effects on humans (Section 11.1.), inhalation of readily available thinners by young adults has been described as a prevalent practice that typically affects the CNS. Inhalation of solvent mixtures containing toluene in the laboratory rat have demonstrated similar effects. Inhalation of a mixture of solvents containing 25% methylene chloride, 5% methanol, 43% heptane, and 23% toluene for 10 minutes (60 to 226 mg/ℓ) caused a decrease in grooming, the appearance of ataxia, abnormal scratching, hind limb flaccid paralysis and, finally, unconsciousness in male Fischer rats. Cumulative effects were noted with 4 intermittent 10-minute exposure periods with 15 minutes between exposures. When the interval between each exposure was increased to 40 minutes, recovery was almost complete (Pryor et al., 1978).

Subchronic exposure to a thinner containing toluene impaired acquisition of a complex behavior. Rats inhaled 50,000 ppm of a readily available commercial paint thinner composed of 42% toluene, 25% methanol, 10% methyl iso-butyl ketone, and minor amounts of other solvents for 4, 8, or 16 weeks (twice-daily for 10 minute periods, 5 days a week) and then were observed for acquisition of temporal discrimination in a differential reinforcement of low rate schedule (DRL 20). In this test, the animal is rewarded for a bar press separated from the last response by 20 seconds. These results suggested that persistent inhalation of thinner vapors impaired temporal discrimination when the animals were tested within a relatively short time after the period of inhalation. However, responses in rats that had a period of rest after exposure did not differ from controls (Colotla et al., 1979).

Studies in laboratory animals have shown that toluene contributes to the symptoms of thinner toxicity. In the studies of Peterson and Bruckner (1978), impairment of cognitive function and muscular coordination were used to monitor CNS depression and narcosis. Behavioral performance (visual placing, grip strength, wire maneuver, tail pinch, and righting reflex) in mice exposed to 3980 ppm toluene for 3 hours decreased over time of exposure, and was inversely correlated with toluene concentration in brain tissue. Concentration of toluene in the brain increased exponentially with the length of exposure and similarly decreased after termination of exposure, as did levels of toluene in liver and blood (Figure 12-1). A single 10-minute exposure to a higher concentration (10,615 ppm) was consistent with the pattern elicited by the lower concentration, longer duration exposure. Recovery of behavioral performance occurred as solvent concentration in the brain decreased after exposure. Bruckner and Peterson (1981a) noted that ataxia, immobility in the absence of stimulation, hypnosis with difficult arousal and unconsciousness were apparent in mice with blood concentrations of 40 to 75 μg/g, 75 to 125 μg/g, 125 to 150 μg/g and >150 μg/g, respectively, as measured by the air bleb method.

A study was conducted by Peterson and Bruckner (1978) with mice to mimic the conditions typical of human solvent-sniffing abuse. Intermittent exposures to 10,615 ppm for approximately 3 hours (5 minutes of exposure followed by 10 minutes without toluene, or 10 minutes of exposure followed by 20 minutes without toluene), or to 11,942 ppm for approximately 3 hours (10 minutes of exposure followed by 20 or 30 minutes without toluene) were conducted. Reflex performance became progressively lower throughout the experimental periods in the regimens that included toluene-free intervals of 20 minutes or less. A 30-minute, toluene-free interval between exposures permitted almost unimpaired performance, indicating complete recovery between exposures (Peterson and Bruckner, 1978).

In a later acute study, Bruckner and Peterson (1981a) exposed mice and rats to seven consecutive cycles. Each cycle consisted of 10-minute inhalation exposure to 12,000 ppm toluene followed by a 20-minute, solvent-free recovery period. Unconditioned performance and reflexes of the animals were tested prior to and following exposure. The mice showed almost complete recovery during the

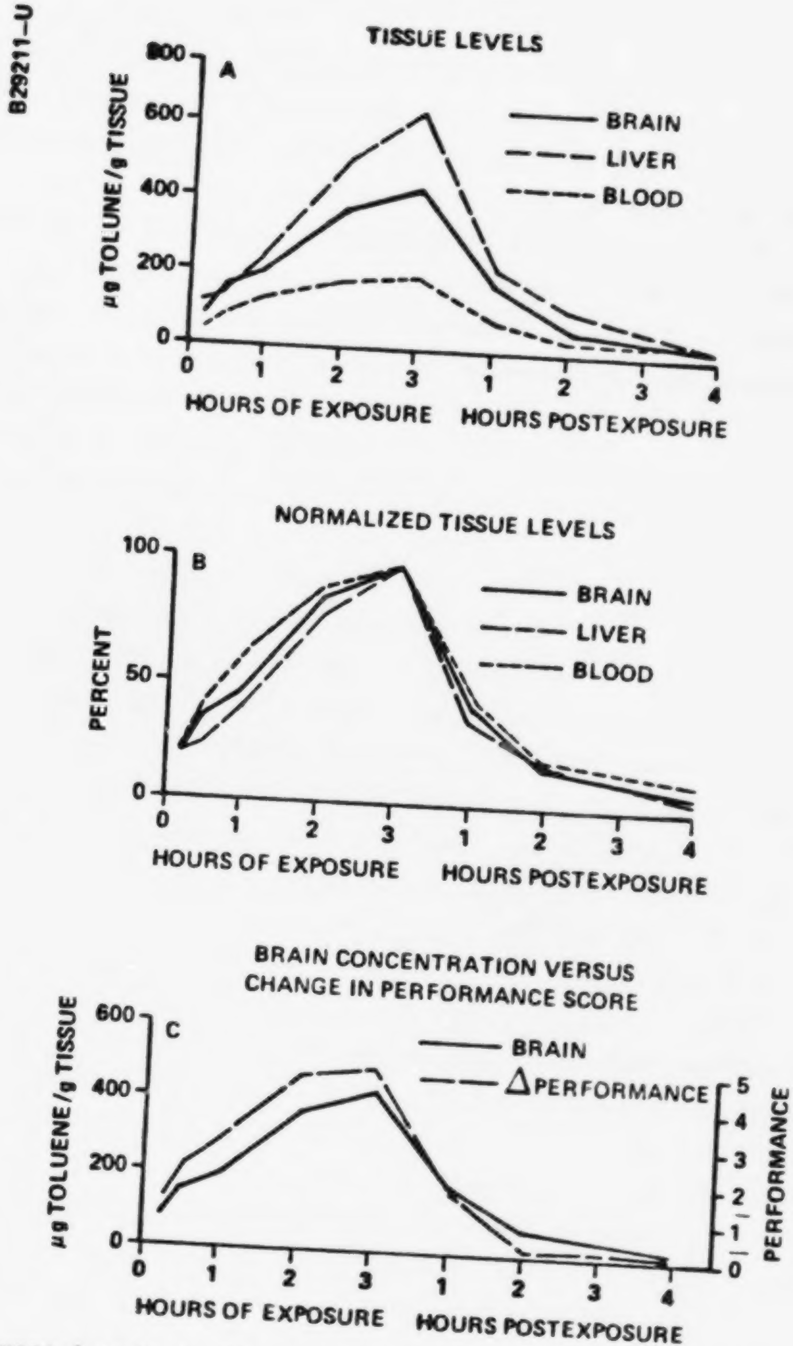

Figure 12-1. Toluene Levels in Tissue and Behavioral Performance (Mice were continuously exposed for 3 hours to an intoxicating concentration of 3980 ppm toluene. Groups of animals were analyzed for air bleb concentration, reflex performance, and tissue levels after 15, 30, 60, 120, and 180 minutes of exposure and 1, 2, and 4 hours postexposure. Figure 12-1A shows toluene levels in liver, brain, and blood. Figure 12-1B shows toluene normalized to the highest mean level in each tissue. Figure 12-1C compares brain levels of toluene with change in performance of the animals. Lines represent means. N = 7 mice on all but 4 hours postexposure, in which case, N = 6. (Peterson and Bruckner, 1978)

course of treatment, while performance scores of rats exhibited a progressive decline. The authors speculate that the rapidity of recovery in mice might be attributed to the higher circulatory, metabolic, and respiratory rates of mice; the increasing CNS depression seen in rats over a 3-hour period of intermittent inhalation might result from a progressive accumulation of the chemical. Substantial residual quantities in the brain 1 hour post exposure had been noted by the same authors in an earlier paper (Bruckner and Peterson, 1981a).

In a subchronic study, groups of 6 mice or 4 rats with comparable numbers of controls were subjected to 7 consecutive cycles (as described in the preceding paragraph) on a daily basis, 5 times a week for 8 weeks (Bruckner and Peterson, 1981a). Depression of body weight gain was observed in both rats and mice during the 8 weeks of intermittent toluene exposure. An increase in SGOT levels was noted in rats and mice, but the increase in mice was not statistically significant. An increase in LDH was seen in rats at all sampling intervals, but this effect was not noted in mice. BUN levels in rats were unaffected by treatment, whereas BUN levels in mice were consistently lower during the period of exposure. Recovery occurred 2 weeks after exposure. There were no effects on brain, lung, liver, heart, or kidney histology, although a depression in gain of organ weights (kidney, brain, lung) was noted in both species.

12.3.2. Effects on Simple and Complex Behavioral Performance.

After a single exposure to 800 ppm toluene for 4 hours, unconditioned reflexes and simple behavior (corneal, grip, and righting reflexes, locomotor activity, and coordination) began to fail (Krivanek and Mullin, 1978; Mullin and Krivanek, 1982). In these studies, male rats were exposed to concentrations of 0, 800, 1600, 3200, and 6400 ppm and tested at 0.5, 1, 2, and 4 hours during exposure and 18 hours after exposure (Table 12-6).

Concentrations of toluene as low as 1 ppm administered 6 hours/day depressed wheel turning performance (a spontaneous activity) after 10 days of exposure in adult male mice (Horiguchi and Inoue, 1977). No effect on body weight was seen at any of the dosages used (1, 10, 100, and 1000 ppm) during 20 daily exposures. However, alterations in blood elements were observed in animals exposed to 10, 100, or 1000 ppm (Table 12-7).

The positive findings at 1 ppm reported by Horiguchi and Inoue (1977), and a report of changes in motor nerve chronaxies in rats exposed continuously to 4 ppm toluene for 85 days (Gusev, 1967; cited by NRC, 1980), are at variance with negative effects observed in other experiments at much higher levels and may be

TABLE 12-6

Behavioral Effects of Toluene

Species	Route	Dose	Effect	Reference
Wistar rats	Inhalation	574, 1148, 2296, and 4595 ppm	Deficit in multiple response schedule	Colotla et al., 1979
Sprague-Dawley rats	Inhalation	150 ppm for 0.5, 1, 2 or 4 h	Initial stimulation followed by depression in multiple response schedule	Geller et al., 1979
Rats (male)	Inhalation	550 to 800 ppm for 4 h/d x 2 wk	No effect on avoidance response	Battig and Grandjean, 1964
Rats	Inhalation	4000 ppm 2 h/d x 60 d	Multiple response schedule No effect on CRF or FR30 Deficit in DRL 12 sec schedule	Ikeda and Miyake, 1978
Rats (male)	Inhalation	3000 ppm for 4 h 1000 ppm for 4 h	Deficit in conditioned avoidance response No effect level	Shigeta et al., 1978
Rats (male)	Inhalation	3200 ppm for 4 h	Deficit conditioned avoidance response	Krivanek and Mullin, 1978; Mullin and Krivanek, 1982
		1600 ppm for 4 h	No-effect-level	
Rats (male)	Inhalation	800 ppm for 4 h	Deficit in unconditioned reflexes and simple behavior	Krivanek and Mullin, 1978 Mullin and Krivanek, 1982
Sprague-Dawley rats	Inhalation	23,000 ppm for 1/2 h/d x 7.6 d	Induced forced turning	Ishikawa and Schmidt, 1973
Mice	Inhalation	3980 ppm for 3 h 10,615 ppm for 10 min	Deficit in visual placing, grip strength, wire maneuver tail pinch, righting reflex	Peterson and Bruckner, 1978
Mice	Inhalation	4,000 ppm for 3 h/d x 5 d/wk for 8 wk	Deficit on an accelerating, rotating bar	Bruckner and Peterson, 1976
Mice (male)	Inhalation	1, 10, 100, 1,000 ppm for 6 h/d x 10 d	Deficit in wheel-turning	Horiguchi and Inoue, 1977
Mice	Inhalation	2650 ppm	Causes mice to fall on side	Faustov, 1958
Mice (male)	I.p.	0.96 g/kg	Loss of righting reflex in 5/7 in 20.6 ± 1.6 min Interval from loss of righting reflex to recovery 35.0 ± 8.2 min. 14.3% lethality in 24 h	Koga and Ohmiya, 1978

h = hour; d = day; wk = week; i.p. = intraperitoneal; min = minute; sec = second.

TABLE 12-7

Myelotoxicity Effects of Toluene

Species	Route	Dose	Effect	Reference
Rats	Inhalation	200, 600, 2500 or 5000 ppm for 7 h/d, 5 d/wk x 5 to 6 wk	A temporary decrease of lymphocytes and total at the highest doses white blood cell count; no anemia; no effect on bone marrow or spleen	Von Oettingen et al., 1942b
Rats, Guinea pigs, Dogs, Monkeys	Inhalation	107 ppm continuous exposure for 90 d, or 1085 ppm for 8 h/d, 5 d/wk x 6 wk	No significant change in leukocyte count, hemoglobin, or hematocrit	Jenkins et al., 1970
Rats	Inhalation	30, 100 or 300 ppm for 6 h/d x 5 d/wk x 24 mo	No effect on any hematological parameter except 2 parameters in females: at 100 or 300 ppm hematocrit was reduced, at 300 ppm mean corpuscular hemoglobin concentration was higher; no histopathology on any organ including spleen and bone marrow	CIIT, 1980
Rats, Dogs	Inhalation	240, 480, or 980 ppm for 6 h/d x 5 d/wk x 65 d	No effect on red blood cell count, white blood cell count, hematocrit, hemoglobin, total and differential white count, SAP, SGPT, SGOT, or BUN; no effect on bone marrow.	Carpenter et al., 1976b

TABLE 12-7 (cont.)

Species	Route	Dose	Effect	Reference
Rats	Inhalation	200, 1000 or 2000 ppm for 8 h/day x 32 wks	Significantly retarded weight gain at 2 higher doses during initial 4 wks; no significant difference in hemoglobin hematocrit and total plasma protein; no significant increase of RBC; significant increase of leucocytes at highest dose at first week of exposure followed by recovery; eosinophile counts decreased rapidly in the first 2 to 4 weeks and then recovered; increase of Mommsen's toxic granules.	Takeuchi, 1969
Rat	Inhalation	112 ppm for 4 h/d x 4 mo	Leukocytosis and chromosome damage in bone marrow	Dobrokhotov and Enikeev, 1975 (cited in U.S. EPA, 1980b)
Rats	Oral	118, 354 or 590 mg/kg/d x 138 d	Normal bone marrow, spleen, bone marrow counts, blood count	Wolf et al., 1956

TABLE 12-7 (cont.)

Species	Route	Dose	Effect	Reference
Rats	Subcutaneous	0.87 g/kg/g x 14 d	Normal leukocyte count, spleen, and bone marrow	Gerarde, 1960
Rat	Subcutaneous	1 g/kg/d x 12 d	11.5% chromosome damaged cells vs. 3.9% in controls	Lyakalo, 1973
Rat	Dermal	10 g/kg/d	Impaired leukopoiesis	Yushkevich and Malypheva, 1975
Mice	Inhalation	1, 10, 100 or 1000 ppm for 6 h/d x 20 d	Leukocytosis at all dose levels; 100, 1000 ppm: depressed red cell count; 10 to 1000 ppm: decreased thrombocyte count; 1000 ppm: trend toward hypoplasia in bone marrow	Horiguchi and Inoue, 1977
Dogs	Inhalation	400 ppm for 7 h/d x 5 d	No change in blood picture; temporary lymphocytosis	Von Oettingen et al., 1942b
Dogs	Inhalation	2000 ppm for 8 h/d x 6 d/wk x 4 mo, and then 2600 ppm for 8 h/d x 6 d/wk x 2 mo	No effect on bone marrow	Fabre et al., 1955

n = number; h = hour; d = day; wk = week; mo = month; SAP = serum alkaline phosphatase; SGPT = serum glutamic pyruvic transaminase; SGOT = serum glutamic oxalacetic transaminase; BUN = blood urea nitrogen.

regarded as spurious. For example, Takeuchi et al. (1981) reported that exposure to 1000 ppm toluene for 16 weeks (12 hours/day) did not produce evidence of peripheral (tail) nerve injury (as determined by nerve condition velocity, mixed nerve conduction velocity, and distal latency measurements) in Wistar rats. Ikeda and Miyake (1978) did not find any effect on spontaneous activity in studies of repeated exposure to 4000 ppm toluene in rats. However, the behavioral tests of the latter authors were carried out 4 days after final exposure, and rapid recovery of behavior after exposure (Shigeta et al., 1978; Peterson and Bruckner, 1978; and Ishikawa and Schmidt, 1973) may explain in part the disparate results.

A single exposure to 3000 ppm toluene for 4 hours disrupted established timing of bar pressing in a conditioned avoidance response test with adult male Wistar rats (Shigeta et al., 1978). Concentrations of 0 and 1000 ppm toluene did not affect this operant behavior. At 3000 ppm, increased response and shortening of the inter-response-interval were noted, but no change in shock counts was seen. Behavioral recovery occurred 1 hour after exposure. Krivanek and Mullin (1978) reported a decrease in conditioned avoidance reflexes in male rats after inhalation of 3200 ppm toluene for 4 hours, but they reported no effect at dose levels of 1600 or 800 ppm.

In another study of operant behavior, Colotla et al., (1979) used rats that had been trained to reinforced bar pressing in a multiple schedule that consisted of fixed ratio (FR) 10 and differential reinforcement of low rates (DRL) 20-second components with a 60-second time out between reinforcement periods. Five trained adult Wistar rats were exposed to concentrations of 574, 1148, 2296, or 4595 ppm toluene, and test sessions were 36 minutes long. Control sessions intervened between solvent exposure sessions to assess recovery. A decrease in rate of response of the FR component and an increase of frequency rate of the DRL component were observed with all doses in a dose-dependent manner. No residual effects were observed.

Exposure to a lower concentration of toluene (150 ppm) for periods of 0.5, 1, 2, or 4 hours affected performance on a multiple fixed ratio-fixed interval schedule of reinforcement in 3 male Holtzman Sprague-Dawley rats. An initial enhancement of FR and FI rates occurred during the shorter exposure periods, followed by a decrease in rates during longer exposure periods (Geller et al., 1979); however, only a small number of animals was used, and the response was not uniform. Battig and Grandjean (1964) found no effect on acquisition or consolidation of an avoidance response in 6 adult male rats after inhalation of toluene that varied in concentration from 550 to 800 ppm, for 4 hours/day for 2 weeks. Continued exposure at similar levels for another week effected a somewhat slower extinction of the avoidance response.

Repeated exposure of rats to inhalation of 4000 ppm toluene, 2 hours daily for 60 days, did not affect spontaneous locomotor activity, emotionality, or learning on 2 operant schedules: memory in a continuous reinforcement schedule (CRF) where every bar press was rewarded by food, and extinction of a fixed ratio (FR 30) schedule performance where every 30th response is reinforced. The exposure impaired learning on a third operant schedule, however, in which acquisition of a differential reinforcement of a low rate of responding (DRL 12 seconds) schedule required rats to allow at least 12 seconds between responses to receive a reward. Impaired performance was present 80 days after final exposure. Exposure to toluene appears to affect more seriously higher levels of cognition. Histological examination of the brain did not reveal any changes (Ikeda and Miyake, 1978).

Inhalation of 4000 ppm toluene by mice for 3 hours/day, 5 times weekly for up to 8 weeks caused a steady deterioration of performance on an accelerating, rotating bar during the initial hour of each session of exposure. Solvent levels in blood and liver increased during each exposure session and decreased quickly after exposure (Bruckner and Peterson, 1976).

Circling (forced turning) was produced within a mean of 7.6 days in 90-day-old male Sprague-Dawley rats (n=10) by repeated exposure to approximately 23,000 ppm (4 to 5 mℓ in 40 to 50 ℓ of air) for one-half hour per day. After 15, 21, or 34 days of recovery, the rats were reexposed daily to toluene. When only 15 days of recovery had elapsed, the number of exposures required to elicit forced turning was significantly less than the number required to acquire the behavior originally. This effect was not seen when a longer period of recovery had elapsed. Thus, toluene has a residual effect and the effect is reversible. This turning was not associated with any histological lesions in the brain (Ishikawa and Schmidt, 1973).

12.3.3. Effect on Electrical Activity of the Brain and Sleep. The effect of toluene on electrical, as well as behavioral, parameters in the brain was studied by Contreras et al. (1979). Twenty cats were exposed via tracheal cannulation to 7000 to 52,000 ppm (approximate) concentrations of toluene for 10-minute periods a day, 7 days a week for 40 days; exposures were started at 7000 ppm and were increased by increments of approximately 7000 ppm (with 10-minute recovery intervals between exposures) each 10 minutes until electrical and behavioral changes appeared. During the first seconds of acute intoxication at 12,000 ppm, the behavior consisted of restlessness, polypnea, coughing, sneezing, and vegetative responses consisting of salivation, mydriasis, and lacrimation. Ataxia appeared 2 minutes later, ending with postural collapse. Changes of electrical activity at this point were found in the anterior lobe of the cerebellum, the amygdala, and the visual cortex. There was no behavioral response to light, sound, or pain stimuli (Table 12-8). The threshold dose for restlessness was approximately 7,000 ppm. No behavioral response to external stimuli occurred at approximately 39,000 ppm. Recovery from ataxia occurred 12 minutes after removal from exposure. With repeated exposure at a concentration of approximately 27,150 ppm, hypersynchronous rhythms spread from the amygdala to the reticular formation, visual cortex, and cerebellum, and electrical activity appeared in the gyrus cinguli which coincided with a display of hallucinatory behavior. These EEG and behavioral signs are similar to complex partial seizures in man (Contreras et al., 1979).

Takeuchi and Hisanaga (1977) found that 1000, 2000, or 4000 ppm toluene administered for 4 hours to groups of 4 or 5 male Wistar rats elicited changes in the sleep cycle, altered cortical and hippocampal EEG rhythms, and increased pulse rates. All phases of sleep were disturbed at concentrations of 2000 and 4000 ppm; 1000 ppm deterred the onset of the slow-wave phase of sleep, but facilitated onset of the paradoxical phase.

A similar observation was made by Fodor et al. (1973), where an increased percentage of REM during sleep was found in female albino rats during exposure to 1000 ppm. A concentration of 1000 ppm decreased cortical and hippocampal components of the EEG (Takeuchi and Hisanaga, 1977). Exposure to 2000 ppm toluene increased cortical fast components and hippocampal components, whereas exposure to 4000 ppm increased the hippocampal fast component as well. At 4000 ppm, excitability measured by rearing reactions (standing on hind legs) increased during the first hour of exposure, but this phase was followed by a depression and the rats were unable to stand or walk; excitability increased again upon reexposure. At 2000 ppm only increased excitability was observed, and at 1000 ppm excitability was not increased significantly. Myoclonic seizures were seen in both 2000 and 4000 ppm treated groups, with greater frequency at the higher concentration.

TABLE 12-8

Central Nervous System Effects of Toluene

Species	Route	Dose	Effect	Reference
Cats	Inhalation	ca. 7,000 to 52,000 ppm 10 min/d x 40 d	Restlessness Autonomic nervous system stimulation, ataxia, collapse EEG changes Seizures	Contreras et al., 1979
Rats	Inhalation	1000, 2000, or 4000 ppm for 4 h	EEG changes Increased excitability Changed sleep cycle Increased pulse rate	Takeuchi and Hisanaga, 1977
Rats (male)	Inhalation	2000 ppm toluene for 8 h/d x 1 wk	Decreased threshold for Bemegride-induced convulsions	Takeuchi and Suzuki, 1975
Sprague-Dawley Rats (male)	Inhalation	500 ppm 6 h/d x 3 d Killed 16 to 18 h after exposure	Increase of catecholamines in lateral palisade zone of median eminence	Andersson et al., 1980
		1000 ppm 6 h/d x 5 d decapitated 4 h after exposures	Increase of catecholamines in subependymal layer of median eminence Increase of FSH	
Rats	Inhalation	1000 ppm x 6 h/d x 6 d/wk x 4 wks	Increased spontaneous activity during light period after repeated exposure. Single exposure did not influence circadian rhythm.	Ikeda et al., 1981
Rats, mice	Inhalation	265 ppm	Threshold affecting CNS	Faustov, 1958

min = minute; d = day; h = hour; wk = week; EEG = electroencephalogram; FSH = follicle-stimulating hormone; CNS = central nervous system.

Convulsion threshold after intraperitoneal injection of Bemegride was decreased significantly by preexposure to 2000 ppm toluene for 8 hours/day in 6 Sprague-Dawley male rats. The change was noted after 1 week of exposure, and convulsion threshold continued to decrease over 6 weeks of exposure. After 8 weeks of exposure, the difference from the controls was not significant, although the convulsion threshold remained lower. The data suggest that toluene renders the CNS more susceptible to induction of a convulsion state. Body weights of these rats were lower than those of controls during the exposure period, although differences were not significant (Takeuchi and Suzuki, 1975).

12.3.4. Effect on Neuromodulators. Andersson et al. (1980) reported an increase of dopamine and noradrenaline in the median eminence after inhalation of 500 ppm and 1000 ppm toluene, respectively, by male rats. The higher levels also produced increases of noradrenaline turnover within the median eminence and the anterior periventricular and paraventricular hypothalamic nuclei. A significant increase of plasma levels of follicle-stimulating hormone (FSH) and a non-significant elevation of prolactin and corticosterone were also noted.

Yamawaki et al. (1982) found a decrease in specific serotonin (^{3}H)-5HT binding to synaptic membrane fractions from whole brains, and from the hippocampus and pons/medulla oblongata regions of rats that were exposed 15 minutes a day to 7000 ppm toluene for 14 days. These results indicate that serotonergic mechanisms may have contributed to some of the observed behavioral effects of exposure (i.e., hindlimb abduction, resting tremor, head weaving).

12.3.5. Minimal Effect Levels. Although most acute as well as chronic studies indicate minor effects of toluene at concentrations under 1000 ppm and most reviews (NIOSH, 1973; U.S. EPA, 1980a; NRC, 1980; Benignus, 1981a, 1981b) have emphasized the negligible effects of toluene on the CNS at this level, several foreign studies suggest that lower level exposures may not be innocuous. Horiguchi and Inoue (1977) found a decrement in simple task performance during exposure to 1 ppm toluene, Gusev (1967) reported lengthened motor nerve chronaxies at 4 ppm, Colotla et al. (1979) noted a decrement in operant behavior at concentrations of 574 ppm, and Anderson et al. (1980) reported histochemical changes in the brain at 500 ppm. In all of these studies, sensitive parameters of CNS activity were measured. Higher concentrations tended to affect more complex tasks. Furthermore, the studies of Andersson et al. (1980) indicate that 500 ppm affects an area of the brain that regulates many vegetative, as well as reproductive, functions. Although the results of the lower exposure level studies (Horiguchi and Inoue, 1977; Gusev, 1967) are inconclusive, these findings indicate that effects of toluene on the CNS at levels below 1000 ppm cannot be totally ignored.

12.4. EFFECTS ON OTHER ORGANS

12.4.1. Blood-Forming Organs. Myelotoxicity is an effect that has been attributed to toluene. Prior to the early 1940's it was believed that toluene had the same effect as benzene; however, in most of the earlier studies toluene was contaminated with benzene. Since then, there have been studies indicating a lack of myelotoxicity, although several have indicated a positive effect (see Table 12-7).

One of the first studies that used pure toluene was that of von Oettingen et al. (1942b). Exposure of rats to 200 to 5000 ppm toluene contaminated with less than 0.01% benzene for 5 to 6 weeks (7 hours/day, 5 days/week) did not affect blood-forming organs, as indicated by the absence of anemia and changes in the bone marrow and spleen. Exposure to the higher concentrations of 2500 and 5000 ppm did produce a daily temporary shift in the blood picture that was characterized by a decrease of lymphocytes and total white blood count, with a moderate increase of segmented cells (Table 12-9). Exposure of dogs to 400 ppm toluene on 5 consecutive days for 7 hours daily produced no appreciable changes in the blood picture, with the exception of a temporary lymphocytosis at the end of exposure (von Oettingen et al., 1942b). Fabre et al. (1955) also found that exposure of dogs to high concentrations of toluene containing less than 0.1% benzene (2000 ppm for 8 hours daily, 6 days weekly for 4 months, and subsequently 2600 ppm for the 2 remaining months) had no effect on the bone marrow.

Wolf et al. (1956) could find no effect on femoral bone marrow, spleen, bone marrow counts, or hematological parameters in female Wistar rats orally dosed with 94.4% pure toluene at levels of up to 590 mg/kg/day for 24 weeks. Exposure of Fischer 344 rats for 24 months (6 hours/day, 5 days/week) to 30, 100, or 300 ppm 99.98% pure toluene did not have any hematological effects (CIIT, 1980) (see Table 12-4); there were also no changes in the bone marrow or spleen.

Male Wistar rats administered a daily subcutaneous dose of 0.87 g/kg body weight for 14 days had a normal leucocyte count, thymus and spleen weight, femoral marrow nucleated cell count, and femoral marrow nucleic acid content (Gerarde, 1956).

TABLE 12-9

Weekly Blood Picture of Normal Rats and Rats Exposed to 600 and 2500 ppm of Toluene 7 Hours/Day, 5 Days/Week, for 5 Weeks

Weeks	Number of Animals	Time	Million red blood cells	g/100 cc hemoglobin	Percent reticulocytes	Thousand white blood cells	Percent mononuclear cells	Percent segmented cells	Thousand total mononuclear	Thousand total segmented cells
NORMAL										
Preexposure period:										
First	5 15	A.M. P.M.	7.0	12.0	3.6	11.9	68	32	8.1	3.8
Second	20	P.M.	6.2	11.3	4.0	16.4	69	31	11.3	5.1
Exposure period:										
First	--	A.M.	--	--	--	--	--	--	--	--
	20	P.M.	6.2	12.0	6.5	17.9	70	30	12.5	5.4
Second	20	A.M.	6.6	11.8	3.6	14.0	65	35	9.1	4.9
	20	P.M.	6.5	10.7	3.9	17.5	64	36	11.2	6.3
Third	20	A.M.	6.7	11.5	4.8	15.9	70	30	11.1	4.8
	20	P.M.	6.2	10.9	4.2	16.2	66	34	10.7	5.5
Fourth	20	A.M.	6.7	12.8	4.4	18.3	73	27	13.4	4.9
	20	P.M.	6.4	11.2	4.7	15.5	65	35	10.1	5.4
Fifth	20	A.M.	6.5	11.5	6.6	17.6	66	34	11.6	6.0
	20	P.M.	6.1	10.1	6.2	18.2	59	41	10.7	7.5
2 Weeks After Exposure	9	A.M.	7.4	13.8	4.7	16.5	68	32	11.2	5.3
	9	P.M.	6.7	12.4	4.6	19.2	66	34	12.7	6.5
600 ppm										
Preexposure period:										
First	15 5	A.M. P.M.	6.8	11.4	3.0	13.1	70	30	9.2	3.9
Second	20	P.M.	--	--	4.6	--	74	26	--	--
Exposure period:										
First	--	A.M.	--	--	--	--	--	--	--	--
	20	P.M.	--	--	5.4	--	78	22	--	--
Second	--	A.M.	--	--	--	--	--	--	--	--
	20	P.M.	--	--	4.6	--	82	18	--	--
Third	--	A.M.	--	--	--	--	--	--	--	--
	20	P.M.	--	--	4.0	--	75	25	--	--
Fourth	20	A.M.	6.5	12.3	4.4	12.2	71	29	8.7	3.5
	20	P.M.	6.3	11.5	3.9	14.5	66	34	9.6	4.9
Fifth	20	A.M.	6.5	11.1	4.8	12.5	71	29	8.9	3.6
	20	P.M.	5.9	10.6	6.2	14.5	65	35	9.4	5.1
2 Weeks After Exposure	10	A.M.	7.2	15.0	5.2	11.0	75	25	8.3	2.7
	10	P.M.	6.8	13.6	5.0	12.3	68	32	8.4	3.9
2500 ppm										
Preexposure period:										
First	10 10	A.M. P.M.	6.8	12.3	4.0	11.0	77	23	8.5	2.5
Second	20	A.M.	6.6	12.1	4.2	13.4	73	27	9.8	3.6
Exposure period:										
First	20	A.M.	6.5	11.6	6.6	16.6	67	33	11.1	5.5
	20	P.M.	6.0	10.4	7.7	12.1	51	49	6.2	5.9
Second	20	A.M.	6.5	11.5	4.6	15.4	70	30	10.8	4.6
	20	P.M.	6.6	10.9	5.2	11.3	55	45	6.2	5.1
Third	20	A.M.	6.4	11.5	4.8	15.9	69	31	11.0	4.9
	20	P.M.	6.5	11.2	4.2	11.3	56.	44	6.3	5.0
Fourth	20	A.M.	6.5	11.8	5.6	14.0	64	36	9.0	5.0
	20	P.M.	6.4	11.0	6.1	12.0	55	45	6.6	5.4
Fifth	20	A.M.	6.0	10.5	5.8	13.3	67	33	8.9	4.4
	20	P.M.	6.5	10.8	5.3	9.9	53	47	5.3	4.6
2 Weeks After Exposure	10	A.M.	7.2	14.4	4.5	15.8	73	27	11.5	4.3
	10	P.M.	5.6	11.7	4.9	11.1	63	37	7.0	4.1

[a] Source: Von Oettingen et al., 1942b

Speck and Moeschlin (1968) noted that subcutaneous injection of 300 or 700 mg/kg pure toluene administered daily to rabbits for 6 and 9 weeks, respectively, had no myelotoxic effects. There were no changes in DNA-synthesis of bone marrow cells as measured by incorporation of ^{3}H-methylthymidine or in peripheral blood elements (leucocytes, thrombocytes, reticulocytes, or erythrocytes).

Braier (1973) reported that subcutaneous injection of 862 mg/kg toluene daily for 6 days produced a moderate depression of granulocytes during the first 2 days of treatment. This was followed by a sharp rise in granulocytes by the end of 6 days (i.e., twice that of the pretreatment level). No significant change was noted in the bone marrow. In contrast, subcutaneous injection of benzene at the same dosage elicited a progressive decrease in granulocyte count throughout the period of treatment. Andrews et al. (1977) found that benzene inhibited the incorporation of ^{59}Fe into erythrocytes of mice, although intraperitoneal injection of toluene alone did not (see Section 15.1).

The studies suggesting a myelotoxic effect include that of Horiguchi and Inoue (1977), who exposed groups of 6 male mice to toluene vapor at concentrations of 1, 10, 100, or 1000 ppm for 6 hours daily over a period of 20 days and found that the 2 highest doses decreased red cell count. Concentrations of 10 ppm and above decreased thrombocyte count. All groups showed an increase in white cell count midway in the study, followed by recovery except in the 100 ppm group. Slight hypoplasia of the bone marrow was noted at the highest dose.

Takeuchi (1969) observed a transient increase in leucocytes in 6 Donryu strain rats exposed to 2000 ppm 99.9% pure toluene containing less than 0.2 ppm benzene in the course of 8-hour daily exposures for 32 weeks, as well as a transient decrease of eosinophile counts upon exposure to 200, 1000, or 2000 ppm toluene under the same regimen (see Table 12-7). After 32 weeks of toluene exposure, all groups including an unexposed control group were subjected to 39 eight-hour daily exposures to benzene prior to sacrifice and histopathological examination. The adrenal weight to body weight ratio was depressed significantly in all groups that had been exposed to toluene. Histologically, the zona glomerulosa of the adrenal cortex of toluene-exposed rats was thicker, while the zona fasciculata and zona reticularis were reduced. The authors suggested that toluene affected the hypothalamo-pituitary-adrenal system. While that hypothesis is tenable, since the rats exposed to toluene differed from the unexposed controls, all groups exposed and unexposed to toluene were also exposed abstract of a later paper (Takeuchi et al., 1972), which was not available for review, noted that exposure of male rats to toluene for 8 hours daily for 4 weeks increased adrenal weight and eosinophil counts and decreased corticosteroid concentration after 1 week.

Topical application of 10 g/kg toluene to rats 4 hours daily for 4 months had no effect on maturation of erythroblasts in the bone marrow, but an increase of plasmic and lymphoid reticular cells in the marrow indicated an impairment of leucopoiesis. A lower dosage of 1 g/kg toluene daily had no effect (Yushkevich and Malypheva, 1975).

Leukocytosis and chromosomal damage in the bone marrow (Section 14.2.3.3.) was noted in rats that had been exposed via inhalation to 112 ppm of toluene, 4 hours daily for 4 months (Dobrokhotov and Enikeev, 1975). Recovery from leucocytosis occurred 1 month after termination of exposure, but the chromosomal damage was unchanged. It was also reported that inhalation of a combination of toluene and benzene produced chromosomal aberrations, which were approximately equal to the sum of aberrations induced by single administration of the solvents. Further, benzene caused leukocytopenia, but the mixture caused leukocytosis. It should be noted, however, that the results of this study should be regarded as inconclusive because Russian reports of toluene-induced chromosomal aberrations have not been corroborated by western investigators (Section 14.2.3.3.).

In the studies by Matsumoto et al. (1971), exposure of Donryu male rats to inhalation of 2000 ppm toluene vapor 8 hours/day, 6 days/week for 43 weeks decreased the ratios of thymus weight to body weight and spleen weight to body weight.

Although the evidence tends to weigh more heavily toward the absence of a myelotoxic effect from toluene exposure in animals, the suggestion made by NRC (1980) that the positive findings may indicate subtle unrecognized hematopoietic responses is sound. For example, the effect of toluene on hematocrit and mean corpuscular hemoglobin concentration in female Fischer rats and not in male rats (CIIT, 1980) is of interest in view of the observation of Hirokawa (1955), where there appears to be a higher susceptibility of the female rabbit to benzene. In that study, the decrease in erythrocytes, hemoglobin content, white blood cells and mean corpuscular hemoglobin concentration, and increase in mean corpuscular volume in the female was simulated in the estradiol propionate-treated orchidectomized male.

There was no increase of erythrocyte fragility in 6 rats that inhaled 20,000 ppm "toluene concentrate" for 45 minutes (Carpenter et al., 1976b). A slight increase in coagulation time was noted in rabbit blood by Fabre et al. (1955) and in rats by von Oettingen et al. (1942b).

12.4.2. Cardiovascular Effects. Several animal studies have shown that massive doses of toluene cause a number of electrocardiographic changes. In addition, a sensitization of the heart to low oxygen levels has been observed.

Inhalation of glue fumes containing toluene for 1 minute significantly slowed sinoatrial heart rate and slightly lengthened the P-R interval in 8 ICR mice. Subjecting the animals to 5 minutes of asphyxia after inhalation of the glue fumes produced a 2:1 atrioventricular block in all animals within an average of 42 seconds of asphyxia. In contrast, after 24 five-minute periods of asphyxia, the sinoatrial heart rate rose, the P-R internal did not lengthen, and atrioventicular (AV) block did not occur in 12 mice (Taylor and Harris, 1970).

In acute inhalation of toluene, atrial fibrillation, bradiarrhythmia, and asystole, along with respiratory paralysis, occurred. Subcutaneous injection of two doses of 0.87 g/kg body weight daily for 6 weeks elicited repolarization disorders, atrial fibrillation, and in some of the rats, ventricular extrasystoles (Morvai et al., 1976).

Intravenous injection of 0.01 mg/kg epinephrine into dogs following inhalation of toluene vapors elicited ventricular fibrillation (Chenoweth, 1946). This observation is of interest, because the "sudden death" syndrome following "glue sniffing" in humans might possibly be explained by an increased secretion of epinephrine, which could cause fibrillation of the heart as a result of the combined effect of the two compounds.

Intravenous injection of 0.5 mg/kg body weight of toluene into rats reduced arterial blood pressure; however, injection of the same dosage by the intraperitoneal or subcutaneous route had no effect on blood pressure (Morvai et al., 1976). No effect on blood pressure was seen in the chronic inhalation study of von Oettingen et al. (1942b), where dogs were exposed to inhalation of 200 to 600 ppm toluene several times weekly for several months. In this study, no effect was observed on blood pressure, heart rate, venous pressure, spinal pressure, respiratory rate, minute volume, or respiratory volume.

12.4.3. Gonadal Effects. Matsumoto et al. (1971) found that exposure of Donyru strain male rats to inhalation of 100 or 200 ppm toluene vapor 8 hours/day, 6 days/week for 1 year produced no change in erythrocyte and leucocyte counts, serum total protein, or cholinesterase activity. However, at the higher dose, degeneration of germinal cells of the testes was found in 4 of 12 animals while normal germinal epithelium was found in controls. Testicular weight was lower than controls at both dose levels. There was a trend toward a decrease of testicular to body weight ratio.

12.5. SUMMARY

The most pronounced effect of toluene in animals is on the CNS. Acute exposure to high levels of toluene has been linked with depression of the CNS, but vapor levels of approximately 1000 ppm appear to have little or no effect on gross manifestations of this parameter. A dose-related response of instability, incoordination, and mild narcosis has been observed in rats exposed daily to toluene vapor at concentrations of 1250 and 1600 ppm, but no effect was noted at 1100 ppm (Batchelor, 1927). Inhalation of 1000 ppm toluene vapor for 4 hours did not increase rearing reactions (standing on hind legs) in rats (Takeuchi and Hisanaga, 1977). Operant behavior (conditioned avoidance response) was unaffected by exposure to 1000 ppm (Shigeta et al. 1978) or 800 ppm (Krivanek and Mullin, 1978) toluene. Inhalation of 1000 ppm for 6 hours/day, 5 days/week for 13 weeks did not produce observable behavioral effects in rats in the pilot study for the chronic CIIT study (CIIT, 1980). Smyth and Smyth (1928) noted that daily inhalation of 1250 ppm for 4 hours each day for 18 days produced narcosis in guinea pigs, while no effect was noted at 1000 ppm during a longer period of exposure. Fabre et al. (1955) found that exposure to 2000 ppm toluene for 8 hours daily, 6 days weekly for 4 months produced only slight nasal and ocular irritation after transient initial hyperactivity in one of two dogs. No behavioral effects were found in rats and dogs after inhalation of 980 ppm "toluene concentrate" (450 ppm toluene) for 6 hours daily for 13 weeks, Carpenter et al. (1976).

The use of more sensitive methods of detection have, however, revealed an effect on simple behavioral parameters and the CNS at lower levels. EEG changes were seen in rats after inhalation of 1000 ppm toluene (Fodor et al., 1973; Takeuchi and Hisanaga, 1977). A deficit was noted in unconditioned reflexes and simple behavior at 800 ppm for 4 hours in rats (Krivanek and Mullin, 1978), in a multiple response schedule at 574 ppm in rats (Colotla et al., 1979), and in wheel-turning in rats at 1 ppm (Horiguchi and Inoue, 1977). Neuromodulator content in the hypothalamus was affected at 500 ppm (Andersson et al., 1980).

Early studies suggested a myelotoxic effect of toluene. However, several studies done since the early 1940's using toluene of greater purity have indicated an absence of toluene-induced injurious effect on blood-forming organs in rats and dogs (von Oettingen et al., 1942a,b; Gerarde, 1959; Wolf et al., 1956; Fabre et al., 1955; Jenkins et al., 1970; Carpenter et al., 1976b; CIIT, 1980). Nonetheless, there is no unanimity on this point. Leukocytosis, impaired leukopoiesis, and chromosomal damage in the bone marrow have been observed in some foreign studies (Horiguchi and Inoue, 1977; Dobrokhotov and Enikeev, 1977; Lyapkalo, 1973; Yushkevich and Malypheva, 1975).

Inhalation of concentrations up to 1085 ppm toluene for 6 weeks or 300 ppm for 24 months, or ingestion of 590 mg toluene/kg body weight for 6 months, produced no liver damage (Svirbely et al., 1944; Carpenter et al., 1976b; Jenkins et al., 1970; CIIT, 1980; Wolf et al., 1956). Exceptions were the studies of von Oettingen et al. (1942b), in which inhalation of 600 ppm toluene caused increases of weight and volume in the liver of rats, Fabre et al. (1955) in which hemorrhagic livers were found in dogs, and Ungvary et al. (1976) in which intraperitoneal injection of 0.43 or 0.82 g/kg toluene produced histological changes in the liver. However, in a more recent study by Ungvary et al. (1980), male CFY rats were exposed by daily inhalation to 265 ppm or 929 ppm analytical grade toluene and female rats were exposed to lower doses five times a week for up to 6 months. No abnormal histological changes were found in the liver, although growth was inhibited at the higher dose in males and at the lower dose in females; specific hepatoxic effects were not noted, although signs of adaptive compensation were observed.

Renal changes consisting of casts in collecting tubules of rats were observed by von Oettingen et al. (1942b) after inhalation of 600 ppm toluene. Hyperemic renal glomeruli and albuminuria were seen in 2 dogs after inhalation of toluene vapors at concentrations of 2000 ppm followed by 2660 ppm for 4 and 2 months, respectively (Fabre et al., 1955). Slight renal degeneration has been observed in guinea pigs (Smyth and Smyth, 1928; Sessa, 1948). No renal damage was found after repeated inhalation of 1085 ppm toluene for 6 weeks in rats, guinea pigs, dogs, or monkeys (Jenkins et al., 1970), after repeated inhalation of 300 ppm for 24 months in rats (CIIT, 1980), or after repeated ingestion of 590 mg toluene/kg body weight for 6 months in rats (Wolf et al., 1956).

Irritative effects were noted in the respiratory tract of dogs, guinea pigs, and rats following exposure to toluene vapor (Browning, 1965; Gerarde, 1959;

Fabre et al., 1955; von Oettingen et al., 1942b; Smyth and Smyth, 1928; Sessa, 1948). Sensitization of the heart after inhalation of toluene has been observed in mice, rats, and dogs (Taylor and Harris, 1970; Morvai et al., 1976; Chenoweth, 1946).

The acute oral LD_{50} of toluene is in the range of 6.0 to 7.5 g/kg in rats (Kimura et al., 1971; Smyth et al., 1969b; Withey and Hall, 1975; Wolf et al., 1956). An acute dermal LD_{50} of 14.1 mg/kg has been determined for rabbits (Smyth et al., 1969b). Slight to moderate irritation of the rabbit and guinea pig skin was elicited by acute or subacute application of toluene (Kronevi et al., 1979; Wolf et al., 1956), and application to the rabbit cornea has caused slight to moderate irritation (Wolf et al., 1956; Smyth et al., 1969; Carpenter and Smyth, 1946).

The inhalation LC_{50} for mice is in the range of 5500 to 7000 ppm toluene for an exposure period of 6 to 7 hours (Svirbely et al., 1943; Bonnet et al., 1979). An LC_{50} of 8800 ppm of "toluene concentrate" for 4 hours (4,038 ppm toluene) was determined for rats (Carpenter et al., 1976b). In guinea pigs, inhalation exposure to 4000 ppm toluene for 4 hours caused death in 2 of 3 animals (Smyth and Smyth, 1928).

Subchronic treatment of rats (von Oettingen et al., 1942b) and rats, guinea pigs, dogs, and monkeys (Jenkins et al., 1970; Smyth and Smyth, 1928) at levels of 200 and 1085 ppm, respectively, did not have a deleterious effect on hematology and organ pathology. Horiguchi and Inoue (1977) did report, however, that mice showed changes in blood elements at levels as low as 10 ppm. Oral administration of toluene at a level of 590 mg/kg/day for 6 months was tolerated by rats with no adverse effects (Wolf et al., 1956).

The only chronic study of toluene was the study performed for CIIT (1980) in which rats were exposed for 24 months via inhalation to toluene at levels of up to 300 ppm. No effect on hematology, clinical chemistry, body weight or histopathology was noted except for two hematologic parameters in females; females exposed to 100 or 300 ppm showed reduced hematocrit levels and increased mean corpuscular hemoglobin concentration at 300 ppm toluene.

12.6. REFERENCES

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13. PHARMACOKINETIC CONSIDERATIONS IN HUMANS AND IN ANIMALS

13.1. ROUTES OF EXPOSURE AND ABSORPTION

For humans, the most common routes of exposure to toluene are through the respiratory tract and the skin. Toluene is absorbed readily through the respiratory tract. In experimental exposures of humans to toluene conducted by Astrand and coworkers (1972; also reported in Astrand, 1975), toluene was detected in arterial blood during the first 10 seconds of exposure. Toluene was supplied in the inspired air at 100 or 200 ppm through a breathing valve and mouthpiece. Unless otherwise specified, in the experiments reported here, human subjects breathed toluene vapor from some type of respiratory apparatus. In resting subjects, the concentration of toluene in arterial blood increased rapidly during the first 10 minutes of exposure and then began to level off, approaching an apparent steady state by 30 minutes. The concentration of toluene in alveolar air (i.e., an air sample taken at the end of a normal expiration) increased concomitantly.

Alveolar and arterial concentrations of toluene were proportional to the concentration in inspired air. At the end of 30 minutes of exposure to 100 or 200 ppm (0.375 or 0.750 mg/ℓ) toluene, the concentration of toluene in alveolar air (mg/ℓ) was 18% of that in inspired air (mg/ℓ), while the concentration in arterial blood (mg/kg) was 270% of that in inspired air (mg/ℓ) (Astrand et al., 1972; Astrand, 1975). The ratio between arterial blood and alveolar air concentrations was 15, which is similar to the *in vitro* blood/air partition coefficients (at 37°C) of 14.6, 15.6, and 15.6 reported for human blood by Sato et al. (1974a), Sherwood (1976), and Sato and Nakajima (1979a), respectively.

According to Veulemans and Masschelein (1978a), subjects' lung clearances (i.e., the virtual volume of inspired air from which all available toluene is absorbed per unit time) decreased during exposure at rest, reaching an apparent steady state 9 to 13 minutes from the beginning of exposure. Lung clearance = $(C_i-C_e)/C_i \times \dot{V}_e$ where C_i is the concentration of toluene in inspired air (mg/ℓ), C_e is the concentration of toluene in expired air (mg/ℓ), and $\dot{V}_e$ is the respiratory minute volume (ℓ/min). Lung clearance varied less among individuals than did the concentration in expired air.

Nomiyama and Nomiyama (1974a) measured the pulmonary retention $((C_i-C_e)/C_i \times 100)$ of volunteers exposed to about 115 ppm toluene for 4 hours. The subjects may have been fairly sedentary because the authors did not mention exercise. Retention at the end of 1 hour was approximately 52% and decreased to 37% at the end of 2 hours, remaining constant at that level for the remaining 2 hours. These results suggest a slower approach to steady-state concentrations in expired or alveolar air than was indicated by the time courses obtained for lung clearance by Veulemans and Masschelein (1978a) or for alveolar air concentrations by Astrand et al. (1972). The results also suggest a lower percentage of uptake or retention than was reported by Veulemans and Masschelein (1978a) and others as will be presented subsequently. The reasons for these discrepancies are unclear.

Exercise affected the absorption of toluene through the respiratory tract. In the experiments of Astrand and coworkers (Astrand et al., 1972; Astrand, 1975), exercise greatly increased the concentrations of toluene in arterial blood and alveolar air of the subjects during exposure, and these concentrations did not level off as soon in exercising subjects as in resting subjects. The concentrations of toluene in arterial blood and alveolar air were approximately the same at 30 minutes of exposure to 200 ppm during rest as at 30 minutes of exposure to 100 ppm during light exercise (50 watts). At 30 minutes exposure to 100 or 200 ppm (0.375 or 0.750 mg/ℓ) toluene, the concentrations in milligrams per liter expressed relative to the concentration in inspired air (mg/ℓ) were 33% for alveolar air and 620% for arterial blood at exercise of 50 watts, and 47% for alveolar air and 725% for arterial blood at exercise of 150 watts. The ratio of arterial to alveolar concentration remained about the same as at rest. Thus, alveolar concentrations appeared to reflect arterial concentrations during exposure to 100 to 200 ppm toluene at rest and various intensities of exercise.

The inhalation of 4% CO_2 by resting subjects during exposure to 100 ppm toluene increased their alveolar ventilation (ℓ/min) and the concentrations of toluene in their arterial blood and alveolar air (Astrand et al., 1972). The increased toluene concentration in blood and alveolar air were similar to those obtained with a corresponding increase in alveolar ventilation during exercise. Because exercise increased both alveolar ventilation and heart rate while CO_2 increased only alveolar ventilation, the effect of exercise on toluene absorption appears to be due to increased alveolar (or pulmonary) ventilation.

In the experiments of Veulemans and Masschelein (1978a), the "steady state" lung clearances of 6 different subjects during exposure to 50 ppm toluene at rest and at workloads of 25 and 50 watts on a bicycle ergometer correlated well

($r^2 = 0.96$) with their respiratory minute volumes. Lung clearance was determined from the regression line to be equal to 0.47 $\dot{V}_e$. The uptake rate in milligrams per minute, which equals lung clearance times the inhaled concentration, therefore was equal to 0.47 $\dot{V}_e C_i$ (where C_i is expressed in mg/ℓ) and total uptake in milligrams equaled 47% of the total amount inhaled. Lung clearances and respiratory minute volumes doubled with an exercise intensity of 25 watts and tripled with an exercise intensity of 50 watts over the corresponding values at rest (Veulemans and Masschelein, 1978a).

Carlsson and Lindqvist (1977) found that the uptake of toluene by 7 male subjects exposed to 100 ppm for 30 minutes (0.375 mg/ℓ) during rest or various levels of exercise (50, 100, and 150 watts on a bicycle ergometer) correlated inversely ($r^2 = 0.72$) with the alveolar concentration determined at the end of 30 minutes exposure, as described by the following equation:

$$\%\ \text{Uptake} = -0.63\ \frac{\text{alveolar concentration (mg/}\ell\text{) x 100}}{\text{inspired concentration (mg/}\ell\text{)}} + 72.9$$

This relationship is logical and applies to other solvents as well (Astrand, 1975; Ovrum et al., 1978). Percent uptake was determined on the basis of the total amount of toluene inhaled and exhaled during the entire exposure period (i.e., the expired air was collected continuously throughout exposure, and thus was a mean value). The uptake ranged from about 47 to 67% at rest and from about 36 to 57% at an exercise level of 150 watts. This group of men comprised 3 thin, 1 slightly overweight, and 3 obese subjects (Carlsson and Lindquist, 1977). Similar uptake values were also more recently reported by Carlsson (1982) for a group of 12 subjects who were exposed to 80 ppm toluene during rest (≈50%), and during a fourth consecutive 30-minute period of 150W exercise (≈30%).

Ovrum and coworkers (1978), monitoring four workers exposed to toluene in a printing plant, found good agreement between the value for percent uptake determined directly from the total amounts of toluene inspired and expired during a sampling period and the value determined indirectly from the instantaneous concentrations in alveolar and inspired air, using the equation given in the preceding paragraph. Percent uptake determined by the direct method was 47% and by the indirect method was 51%. The total uptake of toluene that would occur during exposure to 80 ppm (0.3 mg/ℓ) for an 8-hour work day was calculated using the mean value for pulmonary ventilation of 16 ℓ/min measured for these 4 workers and a percent uptake of 50. The total uptake amounted to approximately 1150 mg (Ovrum et al., 1978).

The percent uptake values determined by Carlsson and Lindquist (1977), Ovrum et al. (1978) and Carlsson (1982) are in reasonable agreement with those previously reported in abstracts from the foreign literature: 54% average uptake during 5 hours' exposure to 271 to 1177 µg/ℓ (Srbova and Teisinger, 1952) and 72% initial retention decreasing to 57% retention towards the end of 8 hours of exposure to 100 to 800 µg/ℓ (Piotrowski, 1967).

Another factor, in addition to exercise, that has been reported to affect the absorption of toluene through the respiratory tract is the amount of adipose tissue in the body (Carlsson and Lindquist, 1977; Carlsson and Lindquist, 1982). Carlsson and Lindquist (1977) found that mean alveolar air concentrations were slightly higher in 3 thin men than in 3 obese men at the end of 30 minutes of exposure to 100 ppm (0.375 mg/ℓ) toluene during rest or exercise. The ranges, however, overlapped. Conversely, the total uptake of toluene during 30 minutes of exposure (determined as previously described) was lower for the thin subjects than for the obese ones (Table 13-1).

TABLE 13-1

Uptake of Toluene in Thin and Obese Men During Exposure to a Toluene Concentration of 375 mg/m^3 (100 ppm)[a,b]

Number of Subjects	Adipose Tissue (kg)	Uptake (mg)			
		Rest	Exercise		
			50 W	100 W	150 W
Thin (N = 3)					
Mean	6.0	61	148	193	228
Range	1.4-10.7	55-69	133-158	168-211	181-271
Slightly overweight (N = 1)	22.8	71	179	246	299
Obese (N = 3)					
Mean	44.0	84	198	258	319
Range	35.1-49.0	72-73	183-206	237-275	258-358

[a] Source: Carlson and Lindquist, 1977

[b] The subjects were exposed during one 30-minute period of rest and three consecutive 30-minute periods of exercise in order of increasing intensity. A 20-minute pause without exposure occurred between rest and exercise. Expired air was collected continuously during exposure.

The thin subjects had a mean adipose tissue content of 6 kg and the obese ones had a mean adipose tissue content of 44 kg. It appears, from Figure 6 in the Carlsson and Lindquist (1977) paper, that the obese men inspired a greater total quantity of toluene than did the thin men. Because the concentrations of toluene in the inspired air were the same for both thin and obese subjects, pulmonary ventilation must have been greater in the obese ones. Thus the differences in uptake between the thin and obese men may have been at least partially due to greater ventilation (respiratory minute volume) in the obese subjects rather than to their adipose tissue per se. Veulemans and Masschelein (1978a) reported finding no correlation between a subject's content of adipose tissue and uptake of toluene during exposures to 50 to 150 ppm toluene lasting about 4 hours. Astrand and coworkers (1972) stated that they found no systematic differences between male subjects (N = 11, adipose tissue 5.7 ± 1.5 kg, mean ± S.D.) and female subjects (N = 4, adipose tissue 13.3 kg, mean; 9.6 to 20.2 kg, range) in alveolar air and arterial blood concentrations of toluene.

Dahlmann and coworkers (1968a, 1968b) investigated the absorption of toluene contained in cigarette smoke through the mouths and respiratory tracts of volunteers. The uptake of toluene from smoke that stayed in the subject's mouth for 2 seconds or less and was not inhaled was 29%; uptake when the smoke was inhaled into the lungs was 93%. It is unclear whether each subject was exposed to a single puff of smoke, the smoke from 1 cigarette (8 puffs), or the smoke from 2 cigarettes.

During inhalation exposure of resting subjects, the concentration of toluene in peripheral venous blood (from the cubital vein of the arm) attained apparent steady state more slowly than did lung clearance or concentrations in alveolar air or arterial blood and was more variable among subjects than were the above mentioned values (Veulemans and Masschelein, 1978a; 1978b; Astrand et al., 1972; Sato and Nakajima, 1978). Peripheral venous concentrations appeared to level off during the second or third hour of exposure. Von Oettingen (1942a, 1942b) had observed that toluene concentrations in subjects' peripheral venous blood at the end of 8 hours of exposure were roughly proportional to the concentrations of toluene (200 to 800 ppm) in the atmosphere of the exposure chamber.

Similar results were obtained when the toluene concentrations in peripheral venous blood of 19 workers at the end of a work week were correlated with toluene concentration in workplace air; the data points showed considerable scatter, but a positive correlation ($r^2 = 0.78$) was observed. (Apostoli et al., 1982). Blood was sampled at the end of the work shift Friday afternoon and air was sampled for 20-25 minutes with personal sampling devices once during the same afternoon. The concentrations of toluene in venous blood ranged from 34 to 572 μg/ℓ and in workroom air from 15 to 182 μg/ℓ (57-686 ppm). The ratio of toluene concentrations in peripheral venous blood (μg/ℓ) to that in air (μg/ℓ) was ≈3. The authors calculated similar values from the data of others for both experimental and occupational human exposure (Astrand et al., 1972; Veulemans and Masschelein, 1978b; Ovrum et al., 1978; Angerer and Behling, 1981).

Veulemans and Masschelein (1978b) reported that the steady-state concentrations of toluene in peripheral venous blood were correlated with the rate of uptake at different inspired concentrations (50, 100, and 150 ppm) ($r^2 = 0.73$) and at different levels of rest and exercise ($r^2 = 0.74$). In both instances, the relationship between peripheral venous concentrations and uptake rate was:

Venous concentration (mg/ℓ) = 0.3 min/ℓ x uptake rate (mg/min).

The concentration of toluene in peripheral venous blood of exercising subjects increased more rapidly and appeared to reach steady-state values sooner than in resting subjects (Astrand et al., 1972; Veulemans and Masschelein, 1978b).

Absorption through the respiratory tract has been studied less extensively in experimental animals than in humans. The initial uptake of a relatively low concentration of toluene was found to be approximately 90% in dogs inhaling toluene (Egle and Gochberg, 1976). Varying the ventilatory rate from 5 to 40 inhalations per minute, the tidal volume from 100 to 250 mℓ, or the concentration of toluene from 0.37 to 0.82 μg/ℓ (approximately 100 to 220 ppm) had no significant effect on the animals' initial respiratory uptake. Toluene was readily absorbed from the upper as well as from the lower respiratory tract. The dogs were anesthetized with sodium pentobarbital for these experiments and breathed toluene from a recording respirometer for 1 to 2 minutes. The percent uptake was calculated from the total amounts of toluene inhaled and exhaled during the 1 to 2 minute exposure.

von Oettingen and coworkers (1942b) found that the concentration of toluene in the peripheral venous blood of dogs at the end of 8 hours of exposure was proportional to the concentration of toluene (200, 400, or 600 ppm) in the air of the exposure chamber. As previously described, similar observations had been made with humans.

Mice exposed singly to an extremely high initial concentration of methyl-^{14}C-toluene in a closed chamber for 10 minutes retained about 60% of the radioactivity when removed from the chamber at the end of the exposure (Bergman, 1979). This value is a rough approximation of absorption because some of the toluene may have been adsorbed to the animals' fur. A substantial portion of the retained dose appears to have been absorbed, however, as shown by its subsequent excretion in the urine (Section 13.4.). The initial concentration of toluene in the chamber (10 µℓ evaporated in a volume of about 30 mℓ, or about 77,000 ppm) would have been above the saturation concentration even if the temperature had been as high as 30°C (saturation concentration = 48,900 ppm at 30°C) (Verschueren, 1977). Bergman (1979) noted that exposure to toluene under these conditions markedly reduced the respiratory rate of the mice and attributed this reduction to irritation. It seems more likely that the decreased respiratory rate was due to narcosis.

Absorption of toluene also occurs through the skin. Dutkiewicz and Tyras (1968a, 1968b), in experiments with humans, measured the absorption of liquid toluene into the skin of the forearm and found the rate of absorption to be 14 to 23 mg/cm^2/hr. This rate was calculated from the difference between the amount of toluene introduced under a watch glass affixed to the skin and the amount remaining on the skin at the end of 10 to 15 minutes. Absorption of toluene from aqueous solutions during immersion of both hands was 160 to 600 µg/cm^2/hr and was directly proportional to the initial concentration of toluene (180 to 600 mg/ℓ). From these results, Dutkiewicz and Tyras (1968a, 1968b) calculated that the absorption of toluene through the skin of both hands during contact with a saturated aqueous solution of toluene for 1 hour could be in the same range as absorption through the respiratory tract during 8 hours of exposure to 26.5 ppm (0.1 mg/ℓ) toluene.

Sato and Nakajima (1978) found, however, that the maximum toluene concentration (170 µg/ℓ) in the blood of subjects who immersed one hand in liquid toluene for 30 minutes was only 26% of the concentration (650 µg/ℓ) in blood of subjects who inhaled 100 ppm toluene vapor for 30 minutes. Blood was collected from the cubital vein of the (unexposed) arm at intervals during and after exposure. Sato and Nakajima (1978) suggested that some of the toluene that penetrates the stratum corneum may be subsequently given off into the air, rather than entering the systemic circulation. Toluene appears to pass slowly from the skin into the bloodstream after penetrating the skin. Guillemin et al. (1974)

reported that the elimination of toluene in alveolar air sometimes increased during the first 20 minutes after the termination of exposure of both hands to liquid toluene, and Sato and Nakajima (1978) noted that the maximum levels of toluene in venous blood were maintained for about 15 minutes after the end of exposure.

Jakobson et al. (1982) monitored the concentration of toluene in the arterial blood of anesthetized guinea pigs following epicutaneous exposure. In this study, a 3.1 cm^2 area of clipped back skin was continuously exposed to liquid toluene by means of a sealed glass ring. It was found that the concentration of toluene in the blood increased rapidly within 1 hour to a peak of ≈1.3 μg/mℓ, and then decreased in spite of the continuing exposure to a plateau concentration of ≈0.5 μg/mℓ after 6 hours. A similar pattern of uptake was observed with other lipophilic solvents (i.e., carbon tetrachloride, hexane, tetrachloroethylene, 1,1,1-trichloroethane, and trichloroethylene).

Absorption of toluene vapor through the skin does not appear to result in a significant contribution to the body burden of toluene as compared to absorption through the respiratory tract. In experiments conducted by Riihimaki and Pfaffli (1978), volunteers wearing light, loose-fitting clothing and respiratory protection were exposed to 600 ppm toluene for 3.5 hours. The subjects remained at rest except for 3 exercise periods, each lasting for 10 minutes, which occurred at 0.5, 1.5, and 2.5 hours of exposure. The exercise was sufficient to stimulate perspiration and raise the skin temperature slightly, conditions which are thought to enhance percutaneous absorption. The concentration of toluene in peripheral venous blood, measured at the end of 1, 2, and 3 hours of exposure, was constant at approximately 100 μg/ℓ.

Riihimaki and Pfaffli (1978) compared total uptake through the skin (calculated from the amount of toluene exhaled assuming that 16% of absorbed toluene is exhaled) with theoretical uptake through the respiratory tract (assuming pulmonary ventilation of 10 ℓ/min and retention of 60%) at the same (600 ppm) level of exposure. They estimated that uptake through the skin was approximately 1% of the theoretical uptake through the respiratory system.

In similar experiments conducted by Piotrowski (1967, reviewed in NIOSH, 1973), subjects exposed dermally to 1600 mg/m^3 (427 ppm) toluene for 8 hours had no increase in urinary excretion of a metabolite (benzoic acid) of toluene. Based on this result, Piotrowski (1967) concluded that absorption of toluene through the skin would not exceed 5% of absorption through the respiratory tract under the same conditions.

The absorption of toluene from the gastrointestinal tract appears to occur more slowly than through the respiratory tract, but appears to be fairly complete based on experiments with animals. The concentration of radioactivity in the blood of adult male rats reached a maximum 2 hours after gastric intubation of 100 µℓ 4-^{3}H-toluene in 400 µℓ peanut oil (Pyykko et al., 1977). The oil may have retarded absorption. Based on the percentages of the dose excreted unchanged in the expired air and as hippuric acid in the urine of rabbits, toluene appears to be completely absorbed from the gastrointestinal tract (El Masri et al., 1956; Smith et al., 1954).

13.2. DISTRIBUTION

Toluene is highly soluble in lipid and sparingly soluble in water, as indicated by the partition coefficients in Table 13-2. Judging from the fluid/air partition coefficients for water, plasma, and blood, much of the toluene in blood may be associated with the lipid and lipoprotein components, including the cellular elements. The tissue/blood partition coefficients for fatty tissues were very high (113 for adipose tissue and 35 for bone marrow); for other tissues, they ranged from about 1 to 3.

Little is known about the tissue distribution of toluene in humans. During inhalation exposure to 50 to 200 ppm toluene, the slow approach to steady-state of peripheral venous concentrations as compared to arterial concentrations (described under absorption) indicates that equilibration with the tissues may take at least 2 to 3 hours. Concentrations in peripheral venous blood do not, however, reflect the discharge of toluene to the tissues as fully as would concentrations in central venous blood. A teenage boy who died from sniffing glue had the following levels of toluene in his tissues: heart blood, 11 mg/kg; liver, 47 mg/kg; brain, 44 mg/kg; and kidney, 39 mg/kg (Winek et al. 1968; also reported in Winek and Collum, 1971).

Several laboratories have investigated the tissue distribution of toluene and its metabolites in animals exposed by inhalation to relatively high concentrations of toluene. The concentrations of toluene in liver, brain, and blood of mice exposed to 3950 ppm (15 mg/ℓ) toluene for 3 hours in a dynamic exposure chamber rose continuously throughout the exposure period, as shown previously in Figure 12-1. Concentrations of toluene reached 625 mg/kg in liver, 420 mg/kg in brain, and 200 mg/kg in blood at the end of exposure (Peterson and Bruckner,

TABLE 13-2

Partition Coefficients for Toluene at 37°C

	Partition Coefficient	Reference
I. Fluid/Air or Material/Air		
Water	2.23	Sato and Nakajima, 1979a
Oil, olive	492	
Blood, Human	15.6	
Fat, human, peritoneal	1296	
Oil, olive	1380	Sherwood, 1976
Lard	1270	
Blood, human	15.6	
Blood, human	14.64	Sato et al., 1974a, 1974b
Blood, rabbit	10.41	
Plasma, rabbit	16.99	
II. Tissue[a]/Blood (Rabbit)		
Liver	2.58	Sato et al., 1974a, 1974b
Kidney	1.54	
Brain	3.06	
Lung	1.92	
Heart	2.10	
Muscle, femoral	1.18	
Bone marow, red[b]	35.43	
Fat, retroperitoneal	113.16	

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[a] Homogenates.

[b] 20% fat by volume.

1978; Bruckner and Peterson, 1981a). Exposure of mice to 10,600 ppm (40 mg/ℓ) toluene for 10 minutes resulted in lower tissue and blood concentrations. Intermittent exposure to 10,600 ppm in cycles of 5 minutes on, 10 minutes off or 10 minutes on, 20 minutes off for a total of 3 hours produced tissue and blood levels approximately 3 times higher than those produced by the single 10-minute exposure to 10,600 ppm and similar to those produced by the 3-hour exposure to 10,600 ppm. The intermittent exposures were an attempt to simulate solvent abuse (e.g., glue sniffing) by humans (Peterson and Bruckner, 1978; Bruckner and Peterson, 1981b).

From an analysis of the time course of toluene levels in central venous blood and in brain of rats during and after whole-body exposure of the animals to 575 ppm toluene, Benignus et al. (1981) concluded that their data fit a one-compartment model. Groups of rats were killed at intervals from 15 to 240 minutes during exposure and at intervals from 15 to 240 minutes after exposure. Blood samples were taken from the posterior vena cava at sacrifice. According to the one-compartment model analysis of Benignus et al. (1981), asymptotes (steady state levels) during exposure to 575 ppm toluene were 10.5 ppm toluene in blood and 18 ppm toluene in brain, and concentrations of toluene in these tissues reached 95% of these estimated asymptotes in approximately 55 minutes. Visual inspection of the experimental data, however, shows that the mean toluene concentrations in the blood and brain of rats exposed for 120 and 240 minutes were above the predicted asymptotes and, particularly in the brain, may still have been increasing appreciably during this interval. The authors mention that this observation "could be construed as an indication that a multicompartment model ought to have been fitted." The elimination of toluene, estimated by one-compartment model analysis, occurred at a slightly faster rate from the brain than from central venous blood. The experimental data of Benignus et al. (1981) for rats are similar to those of Peterson and Bruckner (1978) for mice, previously discussed.

After adult male rats were exposed by inhalation to radioactively-labeled toluene, the highest concentrations of radioactivity were found in their white adipose tissue (Carlsson and Lindquist, 1977; Pyykko et al., 1977). In the experiments of Pyykko and coworkers (1977) the concentration of radioactivity reached a maximum in all tissues, but white adipose tissue within 15 to 30 minutes after the end of 10 minutes of exposure to 4600 ppm 4-^{3}H-toluene. The concentration in white adipose tissue reached a maximum 1 hour after the end of exposure. In the experiments of Carlsson and Lindquist (1977), a similar increase in the concentration of radioactivity in white adipose tissue occurred during the first hour after cessation of exposure for 1 hour to 550 ppm (1.950 mg/ℓ) methyl-^{14}C- toluene. No such increase occurred in other tissues.

Carlsson and Lindquist (1977) found that after white adipose tissue, the next highest concentrations of radioactivity occurred in adrenals and kidneys, followed by liver, cerebrum, and cerebellum. At the end of exposure, white adipose tissue contained a 6-fold higher concentration of radioactivity than did cerebrum or cerebellum. Pyykko et al. (1977) reported that after white adipose tissue, the next highest concentration of radioactivity was found in brown adipose tissue, followed in order of decreasing concentrations by adrenal, stomach, liver and kidney, brain and other tissues, blood, and bone marrow. The loss of radioactivity from adipose tissue and bone marrow appeared to occur more slowly than the loss from other tissues (Pyykko et al., 1977). Radioactivity in the tissues presumably represented toluene and its metabolites.

Bergman (1979), using three-step whole-body autoradiography, investigated the distribution of toluene, its metabolites, and covalently bound reactive intermediates in mice exposed to an extremely high concentration of methyl-^{14}C-toluene. This work was briefly described in a previous report (Bergman, 1978). The mice were exposed singly to a very high initial concentration of toluene for 10 minutes in a closed chamber, as described in Section 13.1., and sacrificed at intervals thereafter. Low temperature autoradiography, performed at -80°C, allowed the detection of both volatile radioactivity (representing toluene) and non-volatile radioactivity (representing metabolites). In a second step, sections were dried and heated to remove volatile material before autoradiography, thus permitting detection of non-volatile metabolites only. In the third step, sections that had been dried and heated were then extracted to remove water-soluble and lipid-soluble radioactivity, presumably leaving only the radioactivity that was covalently bound to proteins and nucleic acids.

Low temperature autoradiography performed immediately after exposure revealed high levels of radioactivity in adipose tissue, bone marrow, and spinal nerves, with some radioactivity also present in the brain, spinal cord, liver, and kidney (Bergman, 1979). Bergman reported that the adrenal did not contain high concentrations of radioactivity, but he did not discuss whether radioactivity was found in the stomach.

The only radioactivity visible in dried, heated sections appeared in the liver, kidney, and blood (Bergman, 1979). This indicates that significant amounts of metabolites had already been formed by the end of exposure, and that the radioactivity in fat and nervous tissue was due to the parent compound. Similarly, as early as 8 minutes after intraperitoneal injection of 290 µg ^{14}C-toluene/kg into mice, the majority of radioactivity in the kidney (78%) and liver (64%) and about half the radioactivity in blood (48%) was reported to represent non-volatile metabolites, while most of the radioactivity in brain and virtually all in the adipose tissue was volatile and thus represented toluene itself (Koga, 1978). The methods used in Koga's study are unclear because the text of the paper is in Japanese, with only the figures, tables, and summary in English. Bergman (1979) reported that no radioactivity was detected in autoradiograms prepared from dried, heated, and extracted sections, indicating an absence of covalent binding.

As had been observed in the studies of Pyykko et al. (1977) and Carlsson and Lindquist (1977), radioactivity disappeared from the tissues relatively quickly after exposure was terminated. The distribution patterns observed in mice killed more than 4 hours after exposure were the same on low temperature autoradiograms as on dried, heated sections. Thus, the radioactivity remaining in the tissues at this time represented non-volatile metabolites. At 8 hours after exposure, only the kidney and the intestinal contents had detectable radioactivity (Bergman, 1979).

Oral administration of 4-^{3}H-toluene (100 µℓ toluene in 400 µℓ peanut oil by intubation) to adult male rats produced a pattern of tissue distribution similar to that produced by inhalation exposure (Pyykko et al., 1977). Distribution appeared to be delayed, however, by absorption from the digestive tract. Maximum tissue concentrations occurred 2 to 3 hours after administration for most tissues and 5 hours after administration for adipose tissue.

In summary, toluene was preferentially accumulated in adipose tissue and was retained longer in adipose tissue and bone marrow than in other tissues, which is reasonable on the basis of the high tissue/blood distribution coefficients of these tissues. Toluene and its metabolites were found in relatively high concentrations in tissues active in its metabolism and excretion (i.e., liver and kidney). Levels in the brain relative to those in other tissues were perhaps lower than would be expected on the basis of the tissue/blood distribution coefficients reported by Sato et al. (1974a, 1974b). Tissue distribution was similar after inhalation and oral exposure.

13.3. METABOLISM

Toluene is thought to be metabolized in humans and in animals by the pathways outlined in Figure 13-1. Some of the absorbed toluene is excreted unchanged in the exhaled air, but the major portion is metabolized by side-chain oxidation to benzoic acid, which is conjugated with glycine to form hippuric acid and then excreted in the urine. Small amounts of benzoic acid may be conjugated with glucuronic acid. Minor amounts of toluene undergo ring hydroxylation, probably via arene oxide intermediates, to form o-cresol and p-cresol, which are excreted in the urine as sulfate or glucuronide conjugates.

Humans exposed to toluene by inhalation exhaled about 16% of the absorbed toluene after exposure was terminated, according to Nomiyama and Nomiyama (1974b) and Srbova and Teisinger (1952, 1953), or 4%, according to Veulemans and Masschelein (1978a). Volunteers inhaling 50 to 150 ppm toluene for about 4 hours during rest or exercise excreted 60 to 70% of the absorbed dose as hippuric acid in the urine during and after exposure (Veulemans and Masschelein, 1979). A similar value was obtained when subjects were exposed to toluene (67 ppm) and xylene (83 ppm) simultaneously for 3 hours; 68% of the absorbed toluene was excreted as urinary hippuric acid during and after exposure (Ogata et al., 1970). Srbova and Teisinger (1953) reported that although most of the benzoic acid in the urine of subjects who inhaled 72 to 532 ppm (0.271 to 2.009 mg/ℓ) toluene was excreted as hippuric acid, 10 to 20% was excreted as a glucuronide conjugate.

The excretion of hippuric acid in the urine was elevated within 30 minutes of the initiation of inhalation exposure, indicating that the metabolism of toluene is rapid (Nomiyama and Nomiyama, 1978; Ogata et al., 1970; Veulemans and Masschelein, 1979). The maximum rate of hippuric acid formation from benzoic acid was reported by Amsel and Levy (1969) to be about 190 μmol/min, and it appeared to be limited by the availability of glycine (Amsel and Levy, 1969; Quick, 1931). Assuming retention of 60% of the inhaled concentration, Riihimaki (1979) estimated that uptake of toluene may saturate the conjugation capacity at a toluene concentration of 780 ppm (32 mmol/m^3) during light work (pulmonary ventilation of 10 ℓ/min) or 270 ppm (11 mmol/m^3) during heavy work (pulmonary ventilation of 30 ℓ/min).

o-Cresol, a compound that is not detected often in normal urine, has been identified in the urine of workers exposed to 7 to 112 ppm toluene (Angerer,

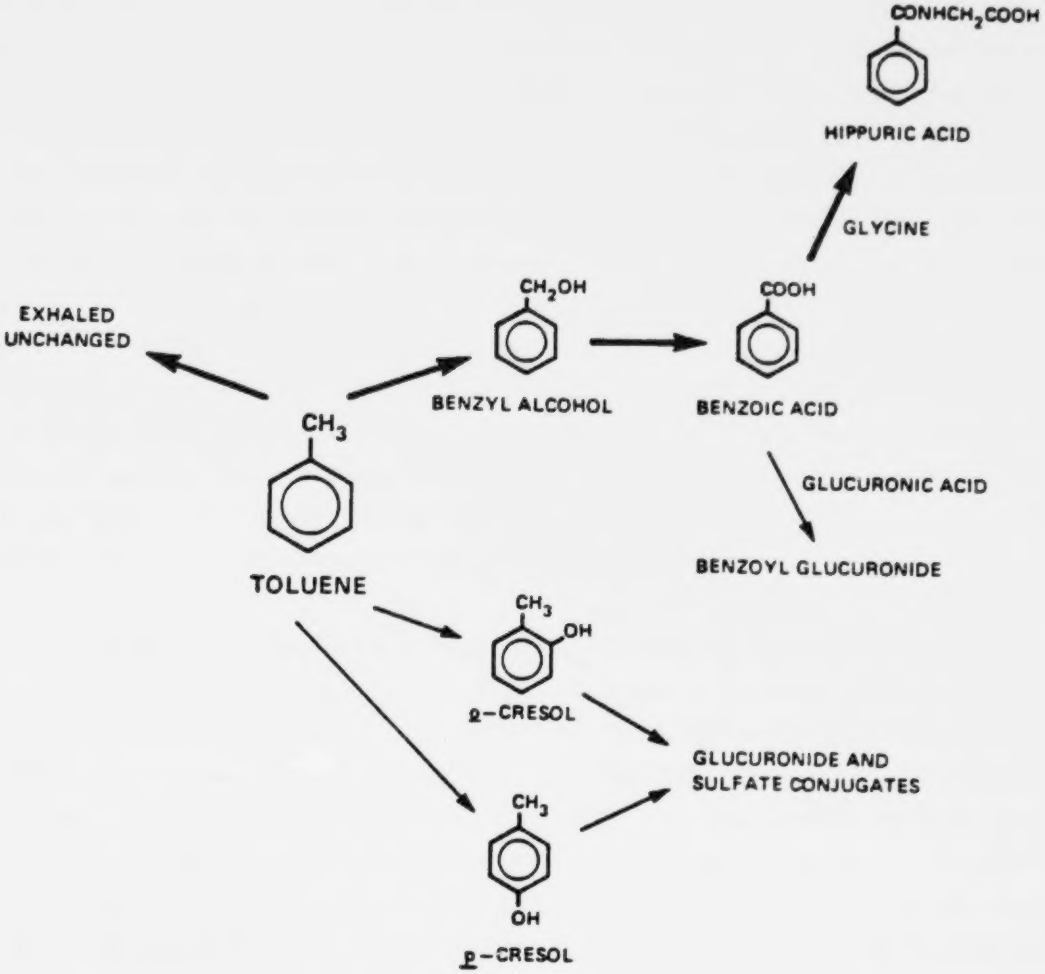

Figure 13-1. Metabolism of Toluene in Humans and Animals (Adapted from Laham, 1970)

1979; Pfaffli et al., 1979; Hansen, 1982). The concentration of o-cresol in urine collected at the end of exposure was directly proportional to the time-weighted average exposure of the workers (Pfaffli et al., 1979). Angerer (1979) estimated that approximately 0.05% of the retained toluene had been metabolized to o-cresol. p-Cresol also may have been a metabolite of toluene, as its concentration was higher in the urine of workers exposed to toluene than in the urine of unexposed workers (Angerer, 1979). The difference, however, was not significant. Wiowode reported finding m-cresol in addition to o- cresol and p-cresol in the urine of workers exposed to 280 ppm toluene (Woiwode et al., 1979) and male subjects who were experimentally exposed to 200 ppm toluene for 4 hours (Woiwode and Drysch, 1981). No m- cresol was detected in the urine of unexposed workers or the subjects before the experimental exposure. No other studies of *in vivo* human or animal metabolism or *in vitro* microsomal metabolism reviewed for this document have detected m-cresol as a metabolite of toluene.

The concentration of phenol has been reported to be slightly elevated in the urine of exposed workers as compared to controls (Angerer, 1979; Szadkowski et al., 1973). The origin of the increased phenol excretion was thought to be the small amount of benzene present in industrially-used toluene (Angerer, 1979).

The metabolism of toluene has been more fully studied in animals than in humans. The initial step in the metabolism of toluene to benzoic acid appears to be side-chain hydroxylation of toluene to benzyl alcohol by the microsomal mixed-function oxidase system. Toluene has been shown to produce a type I binding spectrum with cytochrome P450 from rats and hamsters, indicating that it is probably a substrate for the mixed-function oxidase system (Canady et al., 1974; Al-Gailany et al., 1978). When incubated with rabbit hepatic microsomes, toluene was metabolized primarily to benzyl alcohol (Daly et al., 1968) and small amounts of benzyl alcohol have been detected in the urine of rats given toluene orally (Bakke and Sheline, 1970).

Additional evidence that toluene is metabolized by mixed-function oxidases has been obtained by Ikeda and Ohtsuji (1971) who demonstrated that the induction of hepatic mixed-function oxidases by pretreatment of adult female rats for 4 days with phenobarbital increased the metabolism of toluene. When given 1.18 mg toluene/kg body weight intraperitoneally, phenobarbital-pretreated (induced) rats had greatly elevated urinary excretions of hippuric acid and decreased concentrations of toluene in the blood compared to non-induced rats given the same dose of toluene. Induced rats had high levels of benzoic acid in the blood; non-induced rats had none (blood was obtained at decapitation).

The increased metabolism of toluene by induced rats appeared to reflect an increase in side-chain hydroxylation of toluene, because the activity of hepatic side-chain hydroxylase, assayed _in vitro_ with the model substrate _p_-nitro toluene, was significantly increased per gram of liver. The _in vitro_ oxidation of the resultant alcohol (p-nitrobenzyl alcohol) to the acid (p-nitrobenzoic acid) was not affected. The conjugation of benzoic acid with glycine, measured _in vivo_ as the total amount of hippuric acid excreted after benzoic acid administration, was also unaffected (Ikeda and Ohtsuji, 1971).

It has been assumed (Ikeda and Ohtsuji, 1971; Nomiyama and Nomiyama, 1978; NRC, 1980), by analogy with the metabolism of the model substrate p-nitrotoluene (Gillette, 1959), that benzyl alcohol is metabolized to benzaldehyde by alcohol dehydrogenase and that benzaldehyde in turn is oxidized to benzoic acid by aldehyde dehydrogenase. These enzymes both are found in the soluble fraction from liver. Benzaldehyde itself has not been detected in the urine or expired air of animals given toluene orally (Smith et al., 1954; Bakke and Sheline, 1970). Metabolism of toluene probably occurs primarily in the liver. This assumption is based on the previously discussed tissue distribution of metabolites, the demonstrated metabolism of toluene by liver microsomal preparations, and by analogy with the metabolism of other xenobiotics.

Rabbits intubated with 300 mg toluene/kg body weight eliminated approximately 18% of the dose in the expired air (Smith et al., 1954) and, in another study from the same laboratory, excreted about 74% of the dose as hippuric acid in the urine (El Masri et al., 1956). These results are similar to those obtained with humans who inhaled toluene. None of the toluene appeared to be converted to benzoyl glucuronide (Smith et al., 1954), although about 14% of an oral dose of benzoic acid was excreted by rabbits as the glucuronide conjugate (Bray et al., 1951).

Toluene metabolism appears to be rapid in animals, as shown by the appearance of metabolites in the livers, kidneys, and blood of mice within minutes of exposure to toluene (Bergman, 1979; Koga, 1978) (discussed in Section 13.2.) and by the increased urinary excretion of hippuric acid in rabbits within 0.5 hour of the initiation of inhalation exposure (Nomiyama and Nomiyama, 1978). As was previously mentioned for humans, the rate of conjugation of benzoic acid with glycine may be limited in animals by the availability of glycine. Administration of glycine to dogs exposed by inhalation to 200, 400, or 600 ppm toluene enhanced the rate of hippuric acid excretion (Von Oettingen, 1942b). At the end of 8 hours of exposure to 600 ppm toluene, the concentrations of toluene in peripheral venous blood from glycine-treated dogs were lower than the concentrations in dogs that had not been treated with glycine. No such difference was observed at the two lower exposure levels. This result suggests that conjugation of benzoic acid with glycine may have limited metabolic elimination at the highest level of exposure. The level of exposure at which glycine treatment produced a difference in venous blood levels of toluene is similar to that (780 ppm) calculated by Riihimaki (1979) for saturation of the glycine conjugation capacity of humans.

A minor pathway for the metabolism of toluene is ring hydroxylation by mixed-function oxidases. Incubation of toluene with rat or rabbit liver microsomes resulted in the production of small amounts of o-cresol and p-cresol (Daly et al., 1968; Kaubisch et al., 1972). The migration of deuterium when toluene was labeled in the 4-position and a comparison of the rearrangement products of arene oxides of toluene with the cresols obtained by microsomal metabolism of toluene indicated that arene oxides are intermediates in the metabolism of toluene to o- and p-cresols (Daly et al., 1968; Kaubisch et al., 1972).

Because phenols, including cresols, are eliminated in the urine as sulfate conjugates, thereby increasing the excretion of organic sulfates and decreasing the excretion of inorganic sulfate, investigators have used urinary sulfate excretion after toluene administration as an indicator of cresol formation. Oral doses of 350 mg toluene/kg body weight produced no increase in organic sulfate excretion in rabbits (Smith et al., 1954). In rats, high doses (2.2 and 4.3 g/kg) of toluene, administered orally, resulted in slight but significant decreases in the ratio of inorganic sulfate to total sulfate in the urine, while lower doses did not (Gerarde and Ahlstrom, 1966). This would appear to be a relatively insensitive and nonspecific assay for metabolism to cresols.

Bakke and Sheline (1970) analyzed urinary phenols (after hydrolysis) from male rats placed on purified diets containing neomycin, which reduced the urinary levels of naturally occurring phenols. Toluene, administered orally in a dose of 100 mg/kg body weight, was metabolized to o-cresol (0.04 to 0.11% of the dose) and p-cresol (0.4 to 1.0% of the dose).

Metabolism to cresols is of concern because of the putative arene oxide intermediates, which are highly reactive and may bind to cellular macromolecules. Very little toluene is metabolized via this pathway, however, and the studies already discussed in the distribution section indicate that binding of toluene metabolites to proteins and nucleic acids does not occur to any significant extent.

Van Doorn and coworkers (1980) have reported detecting small amounts of a mercapturic acid, tentatively identified as benzylmercapturic acid (N-acetyl-S-benzyl-L-cysteine), in the urine of male rats treated with toluene. Approximately 0.4 to 0.7% of a dose of 370 mg/kg toluene body weight, administered intraperitoneally, was recovered as the mercapturic acid. The concentration of glutathione in the liver was decreased slightly by administration of toluene. Benzylmercapturic acid would arise from conjugation with glutathione of an electrophilic product of side-chain oxidation of toluene.

The metabolism of toluene appears to result in its detoxification. The length of the sleeping time produced by high doses of toluene (1.18 to 1.45 g/kg intraperitoneally) was decreased in phenobarbital-induced female rats to 50% or less of the sleeping time of controls (Ikeda and Ohtsuji, 1971). Similar results were obtained with male mice (Koga and Ohmiya, 1978). Phenobarbital-induced animals, however, did not have significantly different mortality rates than controls when given high doses of toluene (Ikeda and Ohtsuji, 1971; Koga and Ohmiya, 1978). Male mice given various inhibitors of drug metabolism (SKF 525A, cyanamide, and pyrazole) 30 minutes before the injection of toluene had sleeping times that were significantly longer than those of control mice and had higher mortality rates than did control mice (Koga and Ohmiya, 1978).

13.4. EXCRETION

In both humans and animals, toluene is rapidly excreted as the unchanged compound in expired air and as a metabolite, hippuric acid, in the urine. Most of the absorbed toluene is excreted within 12 hours of the end of exposure.

The concentrations of toluene in exhaled air and in arterial and venous blood of human subjects declined very rapidly as soon as inhalation exposure was terminated (Astrand et al., 1972; Carlsson and Lindquist, 1977; Ovrum et al., 1978; Sato et al., 1974b; Veulemans and Masschelein, 1978a, 1978b). Sato et al. (1974b) reported that semilogarithmic plots of toluene concentrations in alveolar air and in peripheral venous blood versus time after the end of exposure suggested that desaturation occurred in three exponential phases: an initial rapid phase, followed by an intermediate phase, and then a slow phase. The data were obtained from 3 male subjects who inhaled 100 ppm toluene for 2 hours (Sato et al., 1974b; clarified in Sato and Nakajima, 1979b). The desaturation curves were resolved graphically into three components, and constants were determined by the least squares method. The rate coefficients and corresponding half-lives ($t_{1/2}$) for the decay of toluene in peripheral venous blood were 0.355 min^{-1} ($t_{1/2}$ = 1.95 minutes), 0.0197 min^{-1} ($t_{1/2}$ = 35.2 minutes), and 0.00339 min^{-1} ($t_{1/2}$ = 204 minutes). Rate coefficients and half-lives for the decay of toluene in alveolar air were 0.437 min^{-1} ($t_{1/2}$ = 1.59 minutes), 0.0262 min^{-1} ($t_{1/2}$ = 26.5 minutes), and 0.00313 min^{-1} ($t_{1/2}$ = 221 minutes).

Because the rate coefficient for the rapid phase was derived from only two points (at 0 and 5 minutes), the second of which belonged with the intermediate phase, Sato et al. (1974b) noted that the coefficient for the rapid phase involved some error. The data of Sato et al. (1974b) indicate that the decay of toluene concentrations in peripheral venous blood was more gradual than that in expired air. Similar conclusions have been reported by Astrand et al. (1972), and Veulemans and Masschelein (1978b). Astrand et al. (1972) have reported that peripheral venous concentrations declined more gradually than did arterial concentrations.

Veulemans and Masschelein (1978a) and Nomiyama and Nomiyama (1974b) found the excretion curves for toluene in expired air to be adequately described as the sum of 2 exponential terms rather than 3. Subjects for these studies were exposed to 50, 100, or 150 ppm toluene for about 4 hours. The sampling regimens differed from that of Sato et al. (1974b), in that Veulemans and Masschelein (1978a) did not begin monitoring expired air as soon after exposure ended, and Nomiyama and Nomiyama (1974b) sampled expired air infrequently during the period used by Sato et al. (1974b) to determine the first two exponential phases. Rate coefficients for the rapid and slow phases were calculated by Veulemans and Masschelein (1978a) to be 0.340 min^{-1} and 0.00608 min^{-1}, respectively, using a curve-fitting computer program. These rate coefficients corresponded to half-lives of 2.04 and 114 minutes. Nomiyama and Nomiyama (1974b) reported rate coefficients for the rapid phase of 5.10 h^{-1} ($t_{1/2}$ = 8.16 minutes) for men and 3.22 h^{-1} ($t_{1/2}$ = 12.9 minutes) for women; the rate coefficient for the slow phase was 0.335 h^{-1} ($t_{1/2}$ = 124 minutes) for both sexes.

In the desaturation period, men and women expired 17.6 and 9.4%, respectively, of the total amount of toluene calculated to have been absorbed during exposure (Nomiyama and Nomiyama, 1974b). These values are close to what had been reported previously (i.e., 16%) by Srbova and Teisinger (1952, 1953) in abstracts from the foreign literature. Veulemans and Masschelein (1978a) estimated that about 4% of the toluene absorbed during exposure was subsequently excreted in the expired air. Unlike the continuous exposures employed in the other pertinent investigations, however, the exposure regimen employed by Veulemans and Masschelein (1978a) was discontinuous (i.e., four 50-minute periods of exposure separated by 10-minute intervals of nonexposure).

According to Veulemans and Masschelein (1978a) a much greater variability was observed for the excretion of toluene in expired air during the first 4 hours after the end of exposure than had been observed for the related lung clearances during exposure. This variability could be explained partially by differences in respiratory minute volume during the post-exposure period; the percent of absorbed toluene excreted in the expired air during the first 4 hours after exposure correlated positively with respiratory minute volume ($r^2 = 0.71$). Another factor that appeared to affect excretion was the amount of body fat, because there was a significant ($p < 0.025$) negative correlation between fat content as measured by the index of Broca and the percent excretion in expired air after exposure at rest ($r^2 = 0.2134$). This indicates that less of the absorbed toluene would be excreted in the expired air of an obese person than in the expired air of a thin person during the first 4 hours of desaturation. Additionally, subjects who had been exposed to toluene while exercising expired less of the absorbed amount during the first 4 hours of desaturation than did subjects who had been exposed while resting (Veulemans and Masschelein, 1978a).

As previously described, 60 to 70% of the toluene absorbed by humans during inhalation can be accounted for as hippuric acid in the urine (Veulemans and Masschelein, 1979; Ogata et al., 1970). The excretion rate of hippuric acid in the urine of subjects inhaling 50, 100, or 150 ppm toluene increased during the first 2 hours, leveling off at about the third hour after initiation of exposure (Veulemans and Masschelein, 1979; Nomiyama and Nomiyama, 1978). Hippuric acid excretion (mg/hr) declined fairly rapidly after cessation of about 4 hours of exposure. Nomiyama and Nomiyama (1978), treating this decline as a monoexponential process, determined a half-life for hippuric acid in urine of 117 minutes for men and 74 minutes for women. Veulemans and Masschelein (1979) reported an initial, fairly rapid decrease with a half-life between 2.0 and 2.3 hours, followed by a more gradual return to baseline excretion levels by about 24 hours after the start of exposure.

The excretion rate of hippuric acid, measured at the end of about 4 hours of experimental exposure or 8 hours of occupational exposure, correlated reasonably well with the uptake rates (Veulemans and Masschelein, 1979) or total uptake (Wilczok and Bieniek, 1978) during exposure. At a given level of physical activity and exposure concentration, the intra- and interindividual variability in hippuric acid excretion was greater than that noted for uptake rates and was attributed to the variable baseline excretion of this compound because it was not explained by other factors (body weight, body fat, cardiorespiratory parameters) (Veulemans and Masschelein, 1979). Exercise during exposure increased the rate of excretion of hippuric acid (Veulemans and Masschelein, 1979) in accordance with the increase in uptake rate.

Hippuric acid is a normal constituent of urine derived from benzoic acid and precursors of benzoic acid in the diet (Quick, 1931). Concentrations of hippuric acid in the urine of 101 workers not exposed to toluene ranged from 0.052 to 1.271 mg/mℓ (corrected to urine specific gravity of 1.024) and rates of excretion of hippuric acid ranged from 18.47 to 23.00 mg/hr for diuresis of greater than 30 mℓ/hr (Wilczok and Bieniek, 1978). Others have also reported great variability in the physiological concentrations of urinary hippuric acid (Ikeda and Ohtsuji, 1969; Imamura and Ikeda, 1973; Engstrom, 1976; Kira, 1977; Ogata and Sugihara, 1977; Angerer, 1979).

Volunteers exposed in a chamber to 200 ppm toluene for 3 hours followed by a 1 hour break and an additional 4 hours of exposure excreted hippuric acid as shown in Figure 13-2 (Ogata et al., 1970). This exposure regimen was chosen to simulate exposure in the workplace. After leveling off after approximately 3 hours of exposure, excretion increased again during the afternoon exposure. The rate of hippuric acid excretion remained elevated for about 2 hours after exposure was terminated and then declined almost to baseline levels by 18 hours after the end of exposure. The total quantity of hippuric acid excreted during the period lasting 26 hours from the initiation of exposure was directly proportional to the degree of exposure (ppm x time) up through the highest toluene concentration of 200 ppm and could be used to calculate exposure with a fairly high degree of accuracy. Less accurate for this purpose were excretion rates during exposure (i.e., total hippuric acid excreted during exposure ÷ time) and concentrations in urine, corrected for specific gravity. Concentrations of hippuric acid in urine collected during the entire exposure period and corrected to a specific gravity of 1.024 were 0.30 ± 0.10, 2.55 ±

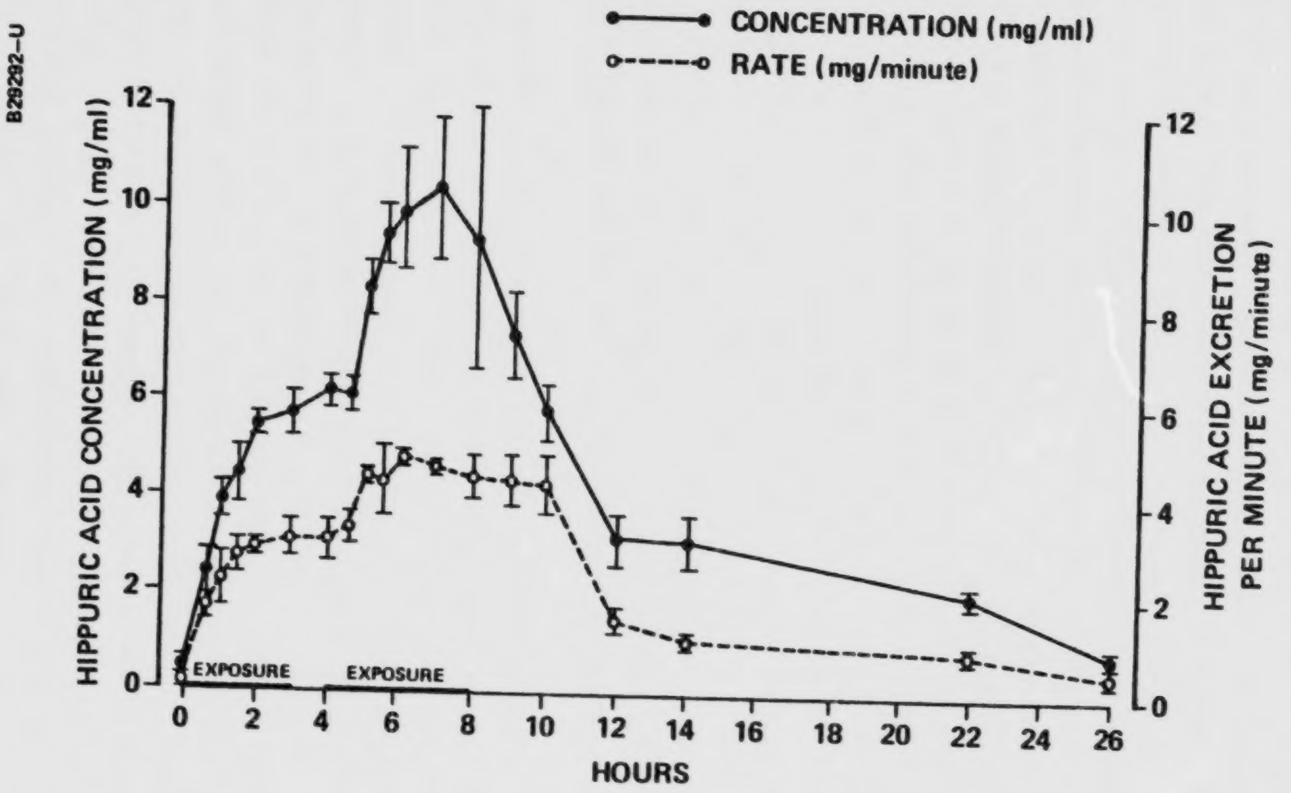

Figure 13-2. Urinary Concentrations and Excretion Rates of Hippuric Acid in Volunteers Exposed to Toluene (Volunteers were exposed to 196 ppm toluene for 3 hours in the morning and for 4 hours in the afternoon with one hour's break in between. Points are means ± SEM.) (Ogata et al., 1970)

0.55, and 5.99 ± 1.20 mg/mℓ (mean ± standard deviation) for control, 100 ppm, and 200 ppm exposed subjects, respectively. Values for controls were lower and more uniform than those reported by others, as described previously.

Spot urine samples collected from workers after at least 3 hours of exposure to toluene (and from nonexposed workers at the same time) have not given as good a distinction between unexposed and exposed workers. Imamura and Ikeda (1973) have pointed out that the upper fiducial limit ($P = 0.10$) of normal hippuric acid concentrations, whether or not corrected for specific gravity, is so close to the lower fiducial limit of workers exposed to 100 ppm toluene (the Threshold Limit Value) that such a measurement would not be reliable in screening for overexposure. This conclusion was based on data reported by Ikeda and Ohtsuji (1969). The correlations between concentrations of toluene in workplace air and the concentration of hippuric acid in urine of individual workers have been relatively poor (Veulemans et al., 1979; Szadkowski, 1973; Ogata et al., 1971). The correlation between exposure concentration and excretion rate during exposure, although slightly better, was also poor: $r^2 = 0.096$ for the correlation with hippuric acid concentration (corrected for specific gravity) and $r^2 = 0.116$ for the correlation with rate of excretion of hippuric acid (Veulemans et al., 1979). Some of the variance in excretion rates was accounted for by differences in lung clearance, and, hence, uptake among workers (Veulemans et al., 1979).

Mice exposed to a very high initial concentration of methyl-^{14}C-toluene in a closed chamber for 10 minutes excreted about 10% of the absorbed dose as volatile material in the exhaled air and about 68% as unidentified compounds in the urine within 8 hours (Bergman, 1979). Details of exposure were discussed in Section 13.1. In these experiments, volatile expired radioactivity (thought to represent the parent compound) was collected continuously in a trapping device. The total volatile radioactivity expired during each time interval was converted to the mean percent dose excreted per minute during that interval and plotted at the end of the interval. The resultant semilogarithmic plot of mean percent dose exhaled per minute versus time was a curve. Computerized non-linear regression analysis of the data according to the method of least squares yielded 3 exponential components with rate coefficients of 0.0659, 0.0236, and 0.0044 min^{-1} corresponding to apparent half-lives of 10.5, 29.4, and 158.7 minutes, respectively.

The respiratory rates of the mice were, according to Bergman (1979), "remarkably reduced" during exposure, and hence probably were reduced during at least part of the post-exposure period. If respiratory minute volumes were also decreased, this would, on the basis of the observations of Veulemans and Masschelein (1978a), be expected to reduce the pulmonary excretion of toluene. The results of Bergman (1979) may therefore not be relevant to exposures at lower concentrations of toluene.

After inhalation exposure of rats or mice to toluene, the disappearance of toluene and its metabolites from blood and from most tissues, including brain, was rapid (Peterson and Bruckner, 1978; Benignus et al., 1981; Carlsson and Lindquist, Pyykko et al., 1977; Bergman, 1979) as described in Section 13.2. The exceptions were white adipose tissue, for which both accumulation and elimination were slow, and bone marrow, for which elimination was very slow (Carlsson and Lindquist, 1977; Pyykko et al., 1977). By 24 hours after exposure to radioactively-labeled toluene, the concentration of radioactivity remaining in most tissues was less than 1% and that remaining in adipose tissue was about 5% of the initial whole-body concentration (Pyykko et al., 1977).

Rabbits exposed to toluene vapor at 350 ppm for 100 minutes or 4500 ppm for 10 minutes had increased rates of urinary hippuric acid excretion that reached maximum values 1.5 hours after exposure (Nomiyama and Nomiyama, 1978). Excretion rates returned to baseline levels at 7 hours after the initiation of exposure to 350 ppm for 100 minutes and at about 3 hours after the initiation of exposure to 4500 ppm for 10 minutes.

Dermal exposure of human subjects to toluene liquid or vapor resulted in the appearance of toluene in the expired air (Guilleman et al., 1974; Riihimaki and Pfaffli, 1978) as discussed in Section 13.1. The excretion of toluene in the expired air of subjects exposed to 600 ppm toluene for 3 hours appeared to consist of at least 2 exponential phases (Riihimaki and Pfaffli, 1978). The mean amount of toluene expired during the "quantitatively significant" portion of the excretion curve was calculated to be 45.9 μmole (4.23 mg) Riihimaki and Pfaffli, 1978). Piotrowski (1967, reviewed in NIOSH, 1973) found that subjects exposed dermally (with respiratory protection) to 427 ppm (1600 mg/m^3) toluene for 8 hours had no detectable increase in urinary excretion of benzoic acid (presumably analyzed after hydrolysis of conjugates).

Oral administration of toluene to rabbits resulted in a pattern of excretion similar to that observed after inhalation exposure of humans. Rabbits (N = 2) intubated with 350 mg toluene/kg body weight expired 18% of the dose as the parent compound within 14.5 hours; less than 1% of the dose was eliminated in the expired air in the period from 14.5 through 35 hours after dosing (Smith et al., 1954). In similar experiments from the same laboratory, rabbits intubated with 274 mg toluene/kg body weight excreted an average of 74% of the dose in the urine as hippuric acid; excretion was complete with 24 hours of dosing (El Masri et al., 1956). The elimination of toluene and its metabolites from tissues and blood of rats given toluene orally (Pyykko et al., 1977) was similar to the pattern already described after inhalation exposure (Pyykko et al., 1977) except that elimination after oral administration appeared to be delayed by a slower rate of absorption than had been observed for inhalation exposure.

The excretion of other metabolites of toluene (i.e., cresols, benzyl alcohol, glucuronide and sulfate conjugates, benzylmercapturic acid) in the urine of humans and animals has already been described in Section 13.3. With the possible exception of benzoylglucuronide (Srbova and Teisinger, 1953), none of these excreted metabolites represented more than about 1% of the total dose of toluene administered or absorbed (Angerer, 1979; Bakke and Sheline, 1970; Van Doorn et al., 1980; Smith et al., 1954). Trace amounts of toluene were eliminated in the urine of humans exposed to toluene (Srbova and Teisinger, 1952).

Biliary excretion of toluene or its metabolites appeared to be negligible. Rats given 50 mg ^{14}C-toluene/kg body weight intraperitoneally excreted less than 2% of the administered radioactivity in the bile within 24 hours (Abou-El-Markarem et al., 1967).

Most of the experimental work on the disposition of toluene in humans and animals has focused on single exposures. The elimination of toluene is rapid enough that few investigators have studied its potential accumulation with repeated daily exposure. Ovrum and coworkers (1978) took samples of capillary blood daily before work from 8 printers exposed occupationally to 35 to 353 ppm toluene. No cumulative increase in blood concentrations of toluene was found during the course of a 5 day work week. Konietzko and coworkers (1980) observed, however, that toluene concentrations in peripheral venous blood tended to increase during the course of a 5 day work week, although the ranges overlapped (Table 13-3). Mean exposure concentrations, measured by a personal air sampling method, did not increase during the week. The blood samples were taken before work on Monday, Wednesday, and Friday from 8 workers exposed to 184 to 332 ppm

TABLE 13-3

Toluene Concentrations in Workplace Air and Peripheral Venous Blood of Exposed Workers[a,b]

		Monday	Tuesday	Wednesday	Thursday	Friday
First week	Toluene in air (ppm)	225 (95-303)	233 (153-383)	209 (107-341)	212 (92-314)	203 (124-309)
	Toluene in blood before exposure (μg/mℓ)	0.12 (0.09-0.24)	--	0.51 (0.28-0.82)	--	0.77 (0.29-1.67)
	Toluene in blood after exposure (μg/mℓ)	3.63 (2.3-4.75)	--	6.69 (4.21-10.36)	---	6.70 (3.99-10.67)
Second week	Toluene in air (ppm)	285 (145-473)	304 (190-521)	309 (213-413)	232 (125-451)	191 (105-432)
	Toluene in blood before exposure (μg/mℓ)	0.27 (0.07-0.57)	--	1.00 (0.35-151)	--	1.21 (0.44-2.29)
	Toluene in blood after exposure (μg/mℓ)	11.60 (6.99-17.10)	--	10.49 (3.24-20.31)	--	5.85 (1.94-9.78)

[a] Source: Konietzko et al., 1980

[b] Means and (range) of eight workers

daily in a plastic processing factory. Concentrations in blood samples taken after work were highly variable and did not seem to follow a consistent pattern.

In an analysis of 3155 samples of urine taken in the course of biological monitoring from different workers on different days of the week and in different workplaces, Lenhert et al. (1978) observed that concentrations of hippuric acid in the urine did not vary with the day of the week except on Monday, when the concentrations were significantly higher than on other days. The authors conjectured that the elevation of hippuric acid concentrations on Mondays was a result of different eating habits on the weekend.

In experiments with dogs, exposure to 400 ppm for 7 hours a day for 5 consecutive days did not result in an increase in the total amount of hippuric acid excreted per day over the period of 5 days or change the time course of urinary excretion (Von Oettingen et al., 1942b). Nor did the concentration of toluene in peripheral venous blood sampled at the end of exposure increase with day of exposure.

13.5. SUMMARY

Toluene is readily absorbed through the respiratory tracts of humans and experimental animals, as would be expected from its blood/air partition coefficient of approximately 15 (Sato and Nakajima, 1979a; Sato et al., 1974a, 1974b; Sherwood, 1976). The amount of toluene absorbed (uptake) is proportional to the concentration in inspired air, length of exposure, and pulmonary ventilation (respiratory minute volume) (Astrand et al., 1972; Astrand, 1975; Veulemans and Masschelein, 1978a).

The uptake of toluene by humans was about 50% of the amount inspired (Veulemans and Masschelein, 1978a; Carlsson and Lindquist, 1977, Ovrum et al., 1978). Total uptake (absorption) can be approximated as follows: Uptake = $0.5\ \dot{V}_e\ C_i\ t$, where $\dot{V}_e$ is the respiratory minute volume in ℓ/min, C_i is the inspired concentration in mg/ℓ, and t is the length of exposure in minutes (Ovrum et al., 1978; Veulemans and Masschelein, 1978a). Because of its dependence on respiratory minute volume, the uptake of toluene is affected by the subjects' level of physical activity (Astrand et al., 1972; Astrand, 1975; Veulemans and Masschelein, 1978a; Carlsson and Lindquist, 1977). A subjects' content of adipose tissue had little or no effect on the uptake of toluene during exposures lasting 4 hours or less (Veulemans and Masschelein, 1978a; Astrand et al., 1972) except in the case of extremely obese individuals (Carlsson and Lindquist, 1977), and even then the increased uptake may have been at least partly due to greater pulmonary ventilation in the obese subjects than in the thin ones. Under "steady state" conditions, peripheral venous concentrations of toluene correlated roughly with exposure concentrations. Inter- and intra-individual variability were high enough to make this an insensitive estimate of exposure concentration or uptake (Von Oettingen et al., 1942a, 1942b; Veulemans and Masschelein, 1978b).

Although toluene appears to be absorbed less readily through the skin than through the respiratory tract, percutaneous absorption of liquid toluene may be significant. The maximum toluene concentration in peripheral venous blood of subjects who immersed one hand in liquid toluene for 30 minutes was about 26% of the concentration in peripheral venous blood of subjects who inhaled 100 ppm toluene vapor for 30 minutes (Sato and Nakajima, 1978). Absorption of toluene vapor through the skin in humans, however, probably amounts to less than 5% of the total uptake through the respiratory tract under the same conditions of exposure (Riihimaki and Pfaffli, 1978; Piotrowski, 1967; reviewed in NIOSH, 1973). Absorption of toluene through the gastrointestinal tract appears to be fairly complete, based on the amounts of toluene and its metabolites excreted by experimental animals after administration of toluene (Pyykko et al., 1977; El Masri et al., 1956; Smith et al., 1954).

Toluene appears to be distributed in the body in accordance with the tissue/blood distribution coefficients and its metabolic and excretory fate. Thus, toluene itself is found in high concentrations in adipose tissue and bone marrow, and toluene and its metabolites are found in moderately high concentrations in liver and kidney (Peterson and Bruckner, 1978; Bruckner and Peterson, 1981a; Carlsson and Lindquist, 1977; Pyykko et al., 1977; Bergman, 1979). The time course of toluene concentrations in the brain appeared to correlate with behavioral effects (Peterson and Bruckner, 1978; Bruckner and Peterson, 1981a).

The major portion of inhaled or ingested toluene is metabolized by side-chain oxidation to benzoic acid, conjugated with glycine to form hippuric acid, and excreted in the urine. Regardless of the route of administration, dose, or species, 60 to 75% of the absorbed (inhalation) or administered (oral) toluene could be accounted for as hippuric acid in the urine (Veulemans and Masschelein, 1979; Ogata et al., 1970; El Masri et al., 1956). Much of the remaining toluene (9 to 18%) was exhaled unchanged (Nomiyama and Nomiyama, 1974b; Srbova and Teisinger, 1952, 1953; Smith et al., 1954). Two percent or less appeared in the urine as cresols and benzylmercapturic acid; these metabolites are of concern because they indicate formation of reactive intermediates that potentially could bind to tissue macromolecules. No evidence of covalent binding to tissue components has been detected, however, by autoradiography of mice that inhaled ^{14}C-toluene (Bergman, 1979).

Most of the toluene absorbed by humans or animals after inhalation or oral exposure is excreted within 12 hours of the end of exposure (Ogata et al., 1970; Veulemans and Masschelein, 1979; Nomiyama and Nomiyama, 1978; Smith et al., 1954; Bergman, 1979). In experimental animals, elimination of toluene and its metabolites from most tissues, including brain, was rapid; elimination from fat and bone marrow was slower (Peterson and Bruckner, 1978; Benignus et al., 1981; Bruckner and Peterson, 1981a; Pyykko et al., 1977; Carlsson and Lindquist, 1977).

In humans, the time course of desaturation after cessation of inhalation exposure appeared to consist of 3 exponential phases with half-lives of 1.95, 35.2, and 204 minutes for toluene concentrations in peripheral venous blood and 1.59, 26.5, and 221 minutes for toluene concentrations in alveolar air (Sato et al., 1974). Toluene concentrations in expired air or peripheral venous blood after the end of inhalation exposure were not reliable indicators of toluene uptake or of exposure concentrations because of the great variability among individuals (Veulemans and Masschelein, 1978a, 1978b; Astrand et al., 1972). Some of this variability, particularly in expired air concentrations, could be explained by differences in exercise load during exposure, in respiratory minute volumes after exposure, and in adipose tissue content (Veulemans and Masschelein 1978a, 1978b). Similarly, although the excretion of hippuric acid in the urine is roughly proportional to the degree of exposure to toluene, inter- and intraindividual variations in the physiological excretion of hippuric acid render quantitation of exposure or uptake from urinary hippuric acid concentration or excretion rates unreliable (Immamura and Ikeda, 1973; Veulemans et al., 1979; Veulemans and Masschelein, 1979; Ogata et al., 1971; Wilczok and Bienick, 1978; and others as reported in Section 13.4.).

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14. CARCINOGENICITY, MUTAGENICITY, AND TERATOGENICITY

14.1. CARCINOGENICITY

CIIT (1980) concluded that exposure to toluene at levels of 30, 100, or 300 ppm for 6 hours/day, 5 days/week for 24 months did not produce an increased incidence of neoplastic, proliferative, inflammatory or degenerative lesions in Fischer 344 male or female rats (see description of study in Section 12.1.2.). Neoplasms were observed frequently in the lungs and liver, endocrine organs, lymphoreticular system, mammary gland, integument, testis and uterus, but the lesions occurred with equal frequency in all control and treatment groups.

Although the CIIT (1980) study was comprehensive and is the only chronic bioassay of toluene presently available, it should be noted that there are several factors that preclude a definite conclusion of non-carcinogenicity. First, it should be noted that this study has been considered inadequate for carcinogenicity evaluation (Powers, 1979) because a maximum tolerated dose (MTD) was not achieved at the highest dose tested in either this 2-year study (300 ppm) or in a preliminary 90-day study (1000 ppm). Also, the low mortality of rats in the CIIT study (14.6%) differs from the mortality rate (up to 25%) normally associated with maintaining these animals under barrier conditions (NCI, 1979a, b). If the CIIT animals were not raised under barrier conditions (which is not stated), then still higher mortality rates would be expected in this age group of Fischer 344 rats. A high spontaneous testicular interstitial cell tumor incidence in aging F344 rats (66 and 85% reported by Coleman et al., 1977 and Mason et al., 1971, respectively) removes this organ from any assessment of carcinogenicity. Additionally, the high spontaneous incidence (16%) of mononuclear cell leukemia on aging F344 rats (Coleman et al., 1977) suggests that this strain may be inappropriate for the study of a chemical that may be myelotoxic (Table 12-7). An independent quality assurance audit of the CIIT study indicated that there were no deviations from protocol requirements, and that the final report accurately reflects (with minor exceptions) the data from the laboratory records. The errors that were noted in the final report do not affect the conclusions drawn from the data.

A chronic bioassay of commercial-grade toluene in rats and mice exposed by inhalation is currently being conducted by the NTP Carcinogenesis Testing Program. Prechronic testing of commercial toluene in the same species exposed by gavage has been completed (NTP, 1983).

Toluene has been utilized extensively as a solvent for lipophilic chemicals being tested for their carcinogenic potential when applied topically to the shaved skin of animals. Results of control experiments with pure toluene have been uniformly negative. Poel (1963), for example, applied toluene (volume not stated) to the shaved interscapular skin 3 times a week throughout the lifetime of 54 male SWR, C3HeB, and A/He mice and found no carcinogenic response. Coombs et al. (1973) treated the dorsal skin of 20 randomly bred albino mice with 1 drop of toluene (6 μℓ) twice a week for 50-weeks. There was no evidence of squamous papillomas or carcinomas in the mice one year following termination of exposure, although survival was only 35% (7 of 20). Doak et al. (1976) applied estimated toluene volumes of 0.05 to 0.1 mℓ/mouse to the backs of CF1, C_3H, and CBaH mice (approximately 25 mice of each sex of each strain) twice weekly for 56 weeks, and failed to elicit skin tumors or a significantly increased frequency of systemic tumors over untreated controls. It is unclear in these studies, however, whether the toluene was applied under an occlusive dressing or allowed to evaporate. Lijinsky and Garcia (1972) did report a skin papilloma in 1 mouse and a skin carcinoma in a second mouse in a group of 30 animals that were subjected to topical applications of 16 to 20 μℓ of toluene twice a week for 72 weeks.

Frei and Kingsley (1968) examined the promoting effect of toluene in Swiss mice following initiation with 7,12-dimethylbenz[a]anthracene (DMBA). In this study, the ears of the mice were topically treated once with 0.1 mℓ of 1.5% DMBA in mineral oil and subsequently, beginning a week later, twice a week with the same volume of 100% toluene for 20 weeks. Results showed that 11 of 35 mice developed tumors (6 permanent, 5 regressing) compared with 8 of 53 negative controls treated with 100% mineral oil (Table 14-1). In 14 mice painted with 100% toluene but no DMBA initiator, 2 developed tumors (1 permanent, 1 regressing). In another study with an identical experimental design, Frei and Stephens (1968) similarly found that 100% toluene promoted a yield of tumors no different from that found in the controls (Table 14-1). In this study, a total of 7 tumors were found in 35 surviving mice treated with toluene following initiation with DMBA; the negative control group (DMBA followed by biweekly applications of mineral oil) had 8 skin tumors in 53 survivors after the 20 weeks.

14.2. MUTAGENICITY

14.2.1. Growth Inhibition Tests in Bacteria. The ability of toluene to induce DNA damage was evaluated in two studies by comparing its differential toxicity to wild-type and DNA repair-deficient bacteria (Fluck et al., 1976; Mortelmans and

TABLE 14-1

Epidermal Tumor Yield in 20 Week Two-Stage Experiments[a]

DMBA	Promoting Agent	No. Surviving Mice	Tumor bearing survivors	Number of Tumors			Tumors per Survivor	Regressing Tumors (%)	Reference
				Permanent	Regressing	Total			
+	None	23[b]	NR	0	0	0	0	0	Frei and Kingsley, 1968
+	5% croton oil[e]	33[b]	NR	381	70	451	13.7	15.5	
+	100% toluene	35[b]	NR	6	5	11	0.31	45.4	
+	100% mineral oil	53[b]	NR	8	0	8	0.15	0	
-	5% croton oil[c]	25[b]	NR	1	2	3	0.11	66.6	
-	100% toluene	14[b]	NR	1	1	2	0.14	5.0	
+	None	23[d]	4%	NR	NR	1	0.04	NR	Frei and Stephens, 1968
+	5% croton oil[c]	33[e]	88%	NR	NR	352	10.7	NR	
+	100%	35[d]	11%	NR	NR	7	0.2	NR	
+	5% croton oil	53[e]	11%	NR	NR	8	0.15	NR	
-	5% croton oil[c]	20[d]	5%	NR	NR	1	0.05	NR	
-	100% toluene	14[d]	0%	0	0	0	0	0	

[a] Ears of Swiss mice treated once with 0.1 mℓ of 0.5% DMBA and subsequently, beginning 1 week later, twice a week with the promoting agent.

[b] Not specifically stated whether this is the number of surviving mice. Also, the number of mice at the start not stated.

[c] In mineral oil

[d] 30 mice at the start

[e] 60 mice at the start

NR = not reported

Riccio, 1980). Two species were tested with negative results: Escherichia coli W3110 (pol A^+) and p3478 (pol A^-) and Salmonella typhimurium SL4525 (rfa) (rec^+) and SL4700 (rfa) (rec^-). In the first study, Fluck et al. (1976) applied toluene (25 μℓ/plate) without metabolic activation directly to wells in the center of culture plates containing the E. coli and found no zones of growth inhibition with either strain. In the Mortelmans and Riccio (1980) study, growth inhibition was also found to be comparable with both the repair competent and deficient strains of the E. coli and S. typhimurium when sterile filter discs inoculated with 0.001 to 0.01 μℓ toluene were placed in the centers of culture plates; these assays were performed both with and without metabolic activation. Mortelmans and Riccio (1980) further found that toluene (0.001 to 0.01 μℓ/plate) was not differentially toxic to either strain of the E. coli or S. typhimurium in quantitative growth inhibition tests. In the quantitative assays, the toluene was preincubated in liquid suspension with the bacteria, with and without S-9 activation, prior to plating; following plate incubation, the numbers of surviving cells were counted (instead of recording measurements of diameters of zones of growth inhibition).

14.2.2. Tests for Gene Mutations

14.2.2.1. ASSAYS USING BACTERIA AND YEAST -- Toluene has been reported to be non-mutagenic in the Ames Salmonella assay when tested with strains TA1535, TA1537, TA1538, TA98, and TA100 (Litton Bionetics, Inc., 1978a; Mortelmans and Riccio, 1980; Nestmann et al., 1980; Bos et al., 1981; Snow et al., 1981), and in the E. coli WP2 reversion to trp^+ prototrophy assay (Mortelmans and Riccio, 1980). The details of these studies are summarized in Table 14-2. All assays were performed in the presence and in the absence of Aroclor 1254-induced rat liver homogenate (S-9) and employed positive and negative controls. It should be noted that there may have been significant losses of toluene from the culture media during incubation in all but one of the aforementioned studies (Snow et al., 1981), particularly at the higher doses tested. Snow et al. (1981) conducted plate incorporation assays in sealed plastic bags and chambers as well as vapor exposures in desiccators to prevent excessive evaporation. The design of the Snow et al. (1981) study is also noteworthy, because the toluene was tested with toluene-induced rat liver S-9 fraction as well as with Aroclor-induced S-9.

Toluene, with and without metabolic activation, was also tested in S. cerevisiae for its ability to induce reversions to isoleucine independence in

TABLE 14-2

Microbial Mutagenicity Assays

Type of Assay	Strain	Metabolic Activation[a]	Dose	Application	Response	Reference
Reverse Mutation						
S. typhimurium	TA 98, 100, 1535, 1537, 1538	yes and no	0.001 to 5.0μℓ/ plate	Plate incorporation	-	Litton Bionetics, Inc., 1978a
S. typhimurium	TA98, 100, 1535, 1537, 1538	yes and no	0.004 to 0.031%[b]	Liquid suspension	-	
S. typhimurium	TA98, 100, 1535, 1537, 1538	yes and no	0.01 to 10μℓ/plate	Plate incorporation	-	Mortelmans and Riccio, 1980
S. typhimurium	TA98, 100, 1535, 1537, 1538	yes and no	5 μℓ/plate	Plate incorporation	-	Nestmann et al., 1980
S. typhimurium	TA98, 100, 1535, 1537, 1538	yes and no	0.115 to 2.3 μℓ/ plate	Plate incorporation	-	Bos et al., 1981
S. typhimurium	TA98, TA100	yes and no[c]	0.3 μl to 100 μℓ/ plate	Plate incorporation[d]	-	Snow et al., 1981
		yes and no[c]	11 to 3764 ppm	Vapor exposure[e]	-	
E. coli	WP2	yes and no	0.01 to 10 μℓ/ plate	Plate incorporation	-	Mortelmans and Riccio, 1980
S. cerevisiae	D7	yes and no	0.001 to 0.5%[f]	Liquid suspension	-	Mortelmans and Riccio, 1980
Mitotic Gene Conversion						
S. cerevisiae	D4	yes and no	0.001 to 5.0μℓ/ plate	Plate incorporation	-	Litton Bionetics, Inc.,
		yes and no	0.138 to 1.1%[b]	Liquid suspension	-	1978a
S. cerevisiae	D7	yes and no	0.001 to 5.0%[f]	Liquid suspension	-	Mortelmans and Riccio, 1980
Mitotic Crossing-Over						
S. cerevisiae	D7	yes and no	0.001 to 5.0%[f]	Liquid suspension	-	Mortelmans and Riccio, 1980

---

[a] Aroclor 1254-induced rat liver homogenate S-9 fraction

[b] 50% mortality at the highest dose

[c] The toluene was tested with toluene-induced S-9 as well as with Aroclor induced S-9.

[d] The plates were incubated in sealed plastic bags or chambers for part of a 72-hr incubation period; in the Aroclor-induced S-9 tests, the plates were removed from the bags after 48 hr, counted, incubated an addition 24 hr, and recounted; in the experiments with toluene-induced S-9 the plates were removed after 24 hr to prevent moisture and spreading problems, and then incubated an additional 48 hr before counting.

[e] The assays were run in a sealed incubation chamber with a second glass plate (open) which contained the toluene; after 24 hr the chambers were opened and the plates incubated for an additional 48 hr.

[f] 100% mortality at 0.1% and 0.5%

strain D7 (Mortelmans and Riccio, 1980), mitotic gene conversion to tryptophan independence in strains D4 (Litton Bionetic, Inc., 1978a) and D7 (Mortelmans and Riccio, 1980), and mitotic crossing over at the ade2 locus in strain D7 (Mortelmans and Riccio, 1980). Toluene did not elicit a positive mutagenic response in any of these tests (Table 14-2).

14.2.2.2. TK MUTATION IN L5178Y MOUSE LYMPHOMA CELLS -- Litton Bionetics, Inc. (1978a) reported that toluene failed to induce specific locus forward mutation in the L5178y Thymidine Kinase (TK) mouse lymphoma cell assay. Toluene was tested at concentrations of 0.05 to 0.30 μℓ/mℓ, with and without mouse liver S-9 activation.

14.2.3. Tests for Chromosomal Mutations

14.2.3.1 MICRONUCLEUS TEST IN MICE -- It was reported recently by SRI International (Kirkhart, 1980) that the intraperitoneal administration of toluene to male Swiss mice failed to cause an increase in micronucleated polychromatophilic erythrocytes in the bone marrow. Doses of 250, 500, and 1000 mg/kg were administered to groups of 32 mice at 0 and 24 hours, with sacrifices 30, 48, and 72 hours after the first dose (8 mice/sacrifice). Five hundred polychromatic erythocytes per animal were evaluated for the presence of micronuclei. The highest dose tested (1000 mg/kg) approximated the LD_{50} for male mice (Koga and Ohmiya, 1978).

14.2.3.2. MOUSE DOMINANT LETHAL ASSAY -- Toluene was recently evaluated for its ability to induce dominant lethal mutations in sperm cells of CD-1 male mice (Litton Bionetics, Inc., 1981). Test mice (12 per dose) were exposed via inhalation to targeted exposure levels of 100 and 400 ppm 6 hours per day, 5 days per week for 8 weeks. Twelve negative control mice were exposed to filtered air in an identical exposure regimen, and 12 positive control mice were injected intraperitoneally with 0.3 mg/kg triethylenemelamine (TEM) on day 40 of the dosing schedule. Following treatment, the males were mated sequentially to 2 females per week for each of 2 weeks; 14 days after the midweek of mating, each female was sacrificed using CO_2 and the number of living and dead implantations were counted. The results of this study showed that toluene did not cause any significant reduction in the fertility of the treated males, and did not cause increases in either pre- or post-implantation loss of embryos when compared with the negative controls. A significant induction of dominant lethal mutations was observed in the positive control mice.

14.2.3.3. CHROMOSOME ABERRATION STUDIES -- Two reports from the Russian literature concluded that toluene induced chromosomal aberrations in rat bone marrow cells following subcutaneous injection (Dobrokhotov, 1972; Lyapkalo, 1973). In an analysis of 720 metaphases from the bone marrow of 5 rats that had been subcutaneously injected with 0.8 g/kg/day toluene for 12 days, Dobrokhotov (1972) found that 78 (13%) showed aberrations. Sixty-six percent of the aberrations were chromatid breaks, 24% were chromatid "fractures", 7% were chromosome "fractures", and 3% involved multiple aberrations. The frequency of spontaneous aberrations in 600 marrow metaphases from 5 control rats injected with vegetable oil averaged 4.16% (65.8% were breaks and 32.4% were chromatid aberrations; no "fractures" or multiple injuries were recorded.). It was further found that similar administration of 0.2 g/kg/day of benzene induced a frequency of chromosomal damage (13.6%) comparable to that of 0.8 g/kg/day of toluene, and that when a mixture of 0.2 g/kg benzene and 0.8 g/kg toluene was injected daily for 12 days, the damage was approximately additive (33.33% aberrations). The significance of the positive clastogenic effects attributed to toluene is difficult to assess, however, because the purity of the sample employed was not stated, and because the distinction between chromatid breaks and fractures is unclear.

Lyapkalo (1973) administered 1 g/kg/day toluene to 6 rats and 1 g/kg/day benzene to 8 rats by subcutaneous injection for 12 days. Treatment with toluene reportedly resulted in chromosome aberrations in 11.6% of the bone marrow cells examined (84 aberrant metaphases/724 cells) compared with 3.87% (40/1033) in olive oil injected controls. The types of aberrations that were observed consisted of "gaps" (60.47%), chromatid breaks (38.37%) and isocromatid breaks (1.16%). Benzene caused a greater degree of chromosome damage than the toluene (57.2% of the cells examined had aberrant chromosomes (573/1002)), and the distribution of aberration types was different (44.72% "gaps", 50.94% chromatid breaks, 4.34% isochromatid breaks). The purity of the toluene used in this study also was not stated.

In a third Russian study, Dobrokhotov and Enikeev (1975) reported that rats exposed to 80 ppm (610 mg/m^3) toluene via inhalation, 4 hours daily for 4 months, showed damaged metaphase chromosomes in 21.6% of the bone marrow cells analyzed. The percentage of metaphases with damaged chromosomes in bone marrow cells from air-exposed control rats was 4.02%. Inhalation of 162 ppm benzene caused damage to chromosomes in 21.56% of the marrow cells, and a mixture of the toluene and benzene (80 and 162 ppm, respectively) damaged chromosomes in an additive manner (41.21% of the cells were involved). Chromosome damage was also observed in all of the groups 1 and 2.5 months after the initial exposure, and 1 month after the end of exposure, the frequency of chromosome damage was still elevated. A total of 96 rats were used in this study, but the number of rats in each group was not stated; it should also be emphasized that the number of cells scored and the purity of the toluene used were not reported.

In contrast to the aforementioned Russian cytogenetics studies, Litton Bionetics, Inc. (1978b) found that intraperitoneal injection of pure toluene into Charles River rats did not induce bone marrow chromosomal aberrations. Toluene was injected at dose levels of 22, 71, and 214 mg/kg in 2 different experiments. In 1 study, 5 rats were sacrificed at 6, 24, and 48 hours following injection of each dose; in a second study, 5 rats were dosed daily at each level for 5 days, and the rats were sacrificed 6 hours after injection of the last dose. Approximately 50 cells per animal were scored for damage. Dimethyl sulphoxide (DMSO; the solvent vehicle) administered intraperitoneally at 0.65 mℓ/rat was used as a negative control, and triethylenemelamine (TEM) in saline at 0.3 mg/kg was used as a positive control. The results of the bone marrow cytogenetic analyses following sacrifice are summarized in Table 14-3. It was also noted that none of the observed aberrations differed significantly in frequency or type from either concurrent or historical spontaneous values.

Gerner-Smidt and Friedrich (1978) reported that toluene at concentrations of 1.52, 152, and 1520 µg/mℓ did not influence the number of structural chromosomal aberrations in cultured human lymphocytes. Benzene and xylene at the same concentrations also had negative clastogenic effects but toluene (152 and 1520 µg/mℓ) and xylene (1520 µg/mℓ) caused a significant cell growth inhibition that was not observed with benzene. The data from this study cannot be adequately evaluated, however, because the source and purity of the toluene were not stated, no positive control experiments were performed, no metabolic activation system was employed, and the type of chromosome damage scored was not specified.

Peripheral blood lymphocytes of toluene-exposed rotogravure workers have also been examined for chromosome aberrations with negative results. In one study, Forni and coworkers (1971) examined the lymphocyte chromosomes from 34 workers from a single plant and 34 controls from outside the plant matched for age and sex. Ten of the workers were exposed daily to minimum concentrations of 131 to 532 ppm benzene for 2 to 7 years and subsequently to toluene in the

TABLE 14-3

Rat Bone Marrow Cell Aberrations Following Intraperitoneal Injection of Toluene[a]

Treatment[b]	Dose	Time of Sacrifice	No. of Animals	Total No. of Cells	Type and Frequency of Aberrations: Structural[d]	Type and Frequency of Aberrations: Numerical	No. of Cells With One or More Aberrations	No. of Animals Without Aberrations	Mitotic Index[e]
DMSO (Solvent)	0.65 mℓ/rat	6 h	5	225	2f,1td	--	3 (1.3%)	3	3.8
		24 h	5	250	--	--	0 (0.0%)	5	6.0
		48 h	5	250	1tb,1f	--	2 (0.8%)	4	6.1
		6 h (SA)[c]	5	227	1td	--	1 (0.4%)	4	5.0
Triethylene Melamine	0.3 mg/kg	24 h	5	250	11tb,2sb,5af,45f, 26t,1r,10td,12>, 1pu,1qr,2ac,3tr	2pp	72 (28.8%)	0	1.4
Toluene	22 mg/kg	6 h	5	250	--	--	0 (0.0%)	5	3.4
		24 h	5	242	--	--	0 (0.0%)	5	5.9
		48 h	5	250	--	--	0 (0.0%)	5	7.0
		6 h	5	238	3f	--	2 (0.8%)	3	6.3
Toluene	71 mg/kg	6 h	5	239	1td	1pp	2 (0.8%)	4	2.5
		24 h	5	227	2td,1af,1f	--	4 (1.8%)	3	4.3
		48 h	3	150	--	--	0 (0.0%)	3	5.7
		6 h	5	212	--	--	0 (0.0%)	5	3.3
Toluene	214 mg/kg	6 h	5	250	1f	2pp	3 (1.2%)	3	4.5
		24 h	5	250	--	1pp	1 (0.4%)	4	3.6
		48 h	5	250	1tb,1td	--	2 (0.8%)	3	5.4
		6 h (SA)[d]	5	250	1td,3af	--	2 (0.8%)	3	5.4

[a] Source: Litton Bionetics, Inc., 1978a

[b] The toluene used was 99.96 wt. % pure (ethylbenzene, 0.03%; p-xylene, <0.01%; m-xylene, <0.01%; sulfur, 0.4 ppm) (Fowle, 1981).

[c] SA = subacute study; rats were dosed daily for 5 days, with sacrifice 6 hours after the last dose

[d] af = acentric fragment (2 tid); f = fragments; pp = polyploid; pu = pulverized chromosome; qr = quadriradial; r = ring; sb = chromosome break; t = translocation; tb = chromatid break; td = chromatid deletion; tr = triradial; > = greater than 10 aberrations

[e] Based on a count of at least 500 cells per animal

general range of 200 to 400 ppm for 14 years; 24 of the workers were exposed only to toluene for 7 to 15 years. (The ink solvent used in this plant was changed from benzene to toluene, which contained some xylene, but reportedly no benzene, after an outbreak of benzene poisoning in 1954.) No significant differences were found between the toluene and control groups in frequencies of stable and unstable chromosome aberrations or in chromosome counts (Table 14-4). Approximately 100 metaphases from each subject or control were scored. The proportion of chromosome changes was significantly higher statistically in the benzene/toluene group compared with controls, and in the benzene/toluene group relative to the toluene group.

Maki-Paakkanen et al. (1980) found no evidence of clastogenicity in cultured peripheral blood lymphocytes from 32 printers and assistants from 2 different rotoprinting factories who had a history of exposure to pure toluene (benzene concentration, $\leq$0.05%; average benzene concentration, 0.006%) at 8-hour, time-weighted average (TWA) concentrations of 7 to 112 ppm. The average age of the workers was 34.2 years and the average length of employment was 14.6 years. Results of analyses showed that when frequencies of chromosome aberrations were compared with those of 15 unexposed research institute workers, there were no significant differences (Table 14-5). Similarly, no significant deviations were observed in the frequencies of aberrations in relation to duration of exposure.

Bauchinger et al. (1982) performed cytogenetic analyses on peripheral lymphocytes from 20 male rotogravure plant workers who were exposed for $\geq$16 years to toluene that contained <0.3% benzene. A group of 24 workers from the same plant with similar age and social background, but without exposure to toluene, served as controls. The continuously measured toluene concentration in the workroom air ranged from 200-300 ppm, and additional small amounts of liquid compound were absorbed through the skin when hands were washed with toluene. The measured concentrations of toluene in blood were reportedly between 0.001 and 0.01%, and there was no exposure to other industrial chemicals. Regular medical examination of these workers during the preceding 10 years indicated no significant adverse neurologic, hematologic, or hepatotoxic effects of exposure (Suhr, 1975) (see Sections 11.1.1.2, 11.2.1, and 11.3, respectively). A significantly larger number of cells with structured chromosome changes was found in the toluene-exposed workers than in the controls (Table 14-6). The aberrations were predominately of the chromatid type, with significantly increased numbers of chromatid breaks and chromatid exchanges; the yield of gaps was also signifi-

TABLE 14-4

Frequency of Unstable and Stable Chromosome Changes and Chromosome Counts in Subjects Exposed to Benzene or Toluene or Both[a]

Expsoure Subjects	No. of Cases	Age Range	Total Cells Counted	% Cells		% Cells With Chromosome Number		
				C_u[b]	C_s[c]	<46	46	>46 (Polyploid)
Benzene (+ toluene)	10	36-54	964	1.66(1.87)[d,e,f]	0.62[e,f]	13.1	86.0	0.9(0.52)
Toluene	24	29-60	2,400	0.80(0.83)[d]	0.08	14.3	85.4	0.3(0.29)
Control subjects	34	25-60	3,262	0.61(0.67)	0.09	10.2	89.5	0.3(0.3)

[a] Source: Forni et al., 1971

[b] Cells with "unstable" chromosome aberrations (fragments, dicentrics, ring chromosomes). The presence of each fragment was considered as one break, the presence of a dicentric or ring chromosome as two breaks.

[c] Cells with "stable" chromosome changes (abnormal monocentric chromosomes due to deletions, translocations, etc., trisomies)

[d] Numbers in parentheses show percentage of calculated breaks.

[e] Difference from toluene group was significant ($P < 0.05$)

[f] Difference from control was significant ($P < 0.01$)

TABLE 14-5

Effect of Occupational Toluene Exposure and Smoking on Chromosomal Aberrations and Sister Chromatid Exchanges[a]

Occupational Toluene Exposure (yr)	No. of Subjects	Mean Age (yr)	Cells Analyzed[b]	Cells with Chromosomal Aberrations (%)				Sister Chromatid Exchanges (SCEs)	
				Gaps Excluded					
				Chromatid Type	Chromosome Type	Total	Gaps Included Total	Cells Analyzed[c]	Mean per Subject per Cell[d]
Total Worker (14.6 yr average exposure)	32	34.2[e]	--	1.0	0.5	1.5	2.5	--	8.5
Total Control	15	34.2[e]	--	0.7	0.9	1.6	2.7	--	8.9
0 (controls)									
Nonsmokers	4	31.0	800	0.5	0.8	1.3	2.3	234	8.0
Smokers	11	35.5	1100	0.9	1.0	1.8	3.1	318	9.7**
Total	15	34.3	1900	0.7	0.9	1.6	2.7	552	9.2
1-10 (mean, 8.0)									
Nonsmokers	3	27.7	300	0.7	0.3	1.0	2.3	79	7.9
Smokers	10	28.2	1000	0.7	0.3	1.0	1.9	295	9.1***
Total	13	28.1	1300	0.7	0.3	1.0	2.0	374	8.8
>10 (mean, 19.3)									
Nonsmokers	11	38.5	1100	0.8	0.5	1.4	2.5	330	7.5
Smokers[f]	8	35.9	800	1.8	0.8	2.5	3.1	205	9.6***
Total	19	37.5	1900	1.2	0.6	1.8	2.8	535	8.3

[a] Source: Maki-Paakkanen et al., 1980

[b] 100 cells analyzed per individual

[c] 30 cells analyzed per individual

[d] Calculated from individual means

[e] Mean value

[f] SCEs were analyzed from 7 subjects. **$p < 0.01$ and *** $P < 0.001$ compared to nonsmokers in the group, one-tailed Student's _t_-test

yr = year

TABLE 14-6

Mean Frequency of Chromosome Abnormalities ± S.E. and SCEs ± S.E. in Lymphocytes of Toluene-Exposed Rotogravure Workers and Unexposed Controls[a,b]

	No. of Subjects	Age (years) I.S.D.	Gaps per cell	S Cells[e] (%)	Aberrations per Cell				SCE per cell
					Chromatid breaks	Chromatid exchanges	Acentric fragments	Dicentrics	
Toluene-Exposed	20[c]	44.2 ± 7.0	0.0248 ± 0.0024[f]	0.90 ± 0.13[g]	0.0036 ± 0.0010[g]	0.0015 ± 0.0005[f]	0.0035 ± 0.0008	0.0005 ± 0.0003	9.62 ± 0.37[h]
Unexposed Controls	24[d]	42.4 ± 10.5	0.019 ± 0.003	0.51 ± 0.06	0.0019 ± 0.0005	0.0004 ± 0.0002	0.0023 ± 0.0004	0.0005 ± 0.0002	8.18 ± 0.25

[a] Source: Bauchinger et al., 1982

[b] 300 cells and 500 cells were analyzed for structural chromosomal changes in each exposed and control subject, respectively. Fifty cells/subject were scored for SCEs.

[c] 11 heavy smokers (>10 cigarettes/day), 1 moderate smoker, and 8 non-smokers

[d] 8 heavy smokers, 1 moderate smoker, and 15 non-smokers

[e] cells with structural chromosome changes (S-cells)

[f] Difference from the unexposed control group was significant ($P<0.05$)

[g] Difference from the unexposed control group was significant ($P\leq0.01$)

[h] The subjects of either group were subdivided into smokers and non-smokers for statistical evaluation. Non-smoking workers had significantly higher ($P=0.02$) SCE values (8.55 ± 0.27) than non-smoking controls (7.75 ± 0.25), and smoking workers had significantly higher ($P=0.020$) SCE values (10.33 ± 0.49) than smoking controls (8.89 ± 0.41).

cantly higher. Although the workers examined in this study were exposed to levels of toluene that are similar to those reported by Forni et al. (1971) and Maki-Paakkanen et al. (1980), it should be noted that a greater number of cells/individual were scored for aberrations (300 or 500 versus 100).

In a report on chromosome aberrations of women in laboratory work, Funes-Craviota et al. (1977) also presented data on 14 workers who were exposed to toluene in a rotogravure factory. Exposures ranged from 1.5 to 26 years and air measurements of toluene showed TWA values of 100 to 200 ppm, with occasional rises up to 500 to 700 ppm; the exposures were sufficient in most cases to elicit frequent headaches and fatigue, and occasional vertigo, nausea, and feelings of drunkenness. The workers had been exposed to toluene since approximately 1950; before 1958, it was stated that the toluene was probably contaminated by a "low" percentage of benzene. Results of lymphocyte analysis showed an excess of chromosome aberrations (abnormal chromosomes and breaks) in the 14 toluene-exposed workers relative to a control group of 42 adults. It should be noted, however, that only a small number of subjects were examined in this study and the exposure background (e.g., extent of exposure to benzene and other chemicals) of the group was not well characterized. The results of this study are presented in Table 14-7. The results of chromosome analyses of 8 other workers with definite exposure to benzene (concentration not measured) for 2 to 10 years prior to 1950, and subsequently to toluene as stated above, are included for comparison.

14.2.3.4. SISTER CHROMATID EXCHANGE -- Gerner-Smidt and Friedrich (1978) reported that in vitro exposure to toluene at concentrations of 15.2, 152, and 1520 μg/mℓ had no effect on the number of sister-chromatid exchanges (SCEs) in cultured human lymphocytes, but no positive control experiments were performed and no metabolic activation system was employed. Twenty-six cells/dose were scored for SCEs and cytotoxicity was observed at the highest dose. Evans and Mitchell (1980) concluded that toluene did not alter SCE frequencies in cultured Chinese hamster ovary (CHO) cells. In the latter study, CHO cells without rat liver S-9 activation were exposed to 0.0025 to 0.04% toluene for 21.4 hours, and CHO cells with activation were exposed to 0.0125 to 0.21% for 2 hours.

In an analysis of cultured peripheral blood lymphocytes from 32 rotogravure workers with daily chronic exposure to 8-hour TWA concentrations of 7 to 112 ppm pure toluene, Maki-Paakkanen et al. (1980) found no increase in SCEs relative to a group of 15 unexposed control subjects. The average age of the workers was 34.2 years and their average length of employment was 14.6 years. The SCE

TABLE 14-7

Chromosome Aberrations in Rotoprinting Factory Workers[a]

	Group[b]		
	Control	Toluene	Benzene/Toluene
No. of Subjects	49	14	8
Age (year)			
Range	0.16 to 63	23 to 54	54 to 65
Mean	24.4	37.2	61.3
No. of Cells Analyzed			
Total	5000	1,400	800
Abnormal			
Total	217	108	76
Frequency range (%)	0 to 20	2 to 15	4 to 17
Mean frequency (%)	4.3	7.7	9.5
No. of Chromosomes Analyzed			
Total	230,000	64,400	36,800
Breaks			
Total	233	124	95
Range (per 100 cells)	0 to 22	2 to 17	6 to 17
Mean (per 100 cells)	5.1	8.9	11.9

[a] Source: Funes-Craviota et al., 1977

[b] Exposure details provided in accompanying text.

analysis was part of a study examining chromosomal aberrations in these workers; the exposure history of the subjects is described in more detail with the summary of the aberration findings (Section 14.2.4.1.), and the results of the SCE analyses are included in Table 14-5.

Bauchinger et al. (1982) reported a significantly increased number of SCEs per peripheral lymphocyte in a group of 20 male rotogravure workers who had been exposed to 200-300 ppm pure (<0.3%) toluene for >16 years (Table 14-6). As described in the more detailed Section 14.2.4.1 summary of this study, chromosomal aberrations were also found in the lymphocytes of these workers.

Funes-Craviota et al. (1977) studied SCE formation in groups of 4 rotogravure printers, 12 laboratory technicians, and 4 children of female laboratory technicians. The printers had been exposed to benzene during the 1940's for 2 to 10 years and subsequently to toluene; exposure to benzene and toluene ranged from 2 to 26 years. TWA concentrations of toluene generally ranged from 100 to 200 ppm (occasionally to 500 to 700 ppm), but benzene concentrations were not measured. The technicians also had a history of exposure to toluene, but the exposures were poorly characterized (duration and concentrations not stated) and each had considerable concurrent exposure to other solvents as well, particularly benzene and chloroform. Results of peripheral lymphocyte analysis (20 cells/individual scored) showed a statistically significant increase in SCEs in the laboratory technicians and the children of female technicians, but not in the exposed printers; however, due to the nature of the exposure, the increases noted cannot be exclusively attributed to toluene.

14.3. TERATOGENICITY

14.3.1. Animal Studies. Toluene was reported in a recent abstract to be teratogenic to CD-1 mice following oral exposure (Nawrot and Staples, 1979). Toluene was administered by gavage from days 6-15 of gestation at levels of 0.3, 0.5, and 1.0 mℓ/kg/day (approximately 0.26, 0.43, and 0.87 g/kg/day, respectively) and from days 12 to 15 at 1.0 mℓ/kg/day. The vehicle used was cottonseed oil (0.5% of maternal body weight per dose). A significant increase in embryonic lethality occurred at all dose levels when administered on days 6 to 15, and a significant reduction in fetal weight was measured in the 0.5 and 1.0 mℓ/kg groups. Exposure to 1.0 mℓ/kg toluene on days 6 to 15 also significantly increased the incidence of cleft palate; this effect reportedly did not appear to be due merely to a general retardation in growth rate. When toluene was administered at 1.0 mℓ/kg on days 12 to 15, however, decreased maternal weight gain was the only effect observed. Maternal toxicity was not noted after exposure to toluene on days 6 to 15 at any dose level. It should be emphasized that the numbers of mice exposed and the numbers of fetuses examined were not stated in the available abstract of this study; a complete copy of this report is not available for review but has been submitted for publication.

Hudak and Ungvary (1978) recently concluded that toluene was not teratogenic to CFLP mice or CFY rats when administered via inhalation according to the following schedule:

	Dose	Days of Pregnancy	Duration
CFPL mice	133 ppm (500 mg/m^3)	6-13	24 hours/day
	399 ppm (1500 mg/m^3)	6-13	24 hours/day
CFY rats	266 ppm (1000 mg/m^3)	1-21	8 hours/day
	399 ppm (1500 mg/m^3)	1-8	24 hours/day
	399 ppm (1500 mg/m^3)	9-14	24 hours/day

It was found that the entire group of mice exposed to 399 ppm toluene died within 24 hours. Toluene administered to rats at 399 ppm also had an effect on maternal survival, but none of the exposures adversely affected the incidence of external or visceral malformations in either species relative to air-exposed controls (Table 14-8). An increased incidence of skeletal anomalies (fused sternebrae, extra ribs) was observed, however, in the rats exposed continuously to 399 ppm toluene on days 9 to 14, and signs of retarded skeletal development (including poorly ossified sternebrae, bipartite vertebra centra, and shortened 13th ribs) were found in the rats exposed on days 1 to 8 (399 ppm) and during the entire period of pregnancy (days 1 to 21) at 266 ppm for 8 hours/day. An embryotoxic effect of toluene was further indicated by low fetal weights in the mice, and in the rats exposed on days 1 to 8 of pregnancy. Fetal loss (percent of total implants), mean litter size, mean placental weight, and maternal weight gain were unaffected by exposure in either species.

In a more recent teratogenicity study, groups of 20 CFY rats were exposed to 266 ppm (1000 mg/m^3) toluene, 125 ppm (400 mg/m^3) benzene, or a combination of these concentrations of toluene and benzene vapor for 24 hours/day on days 7 to 14 of gestation (Tatrai et al., 1979). A group of 22 rats inhaling pure air

TABLE 14-8

Teratogenicity Evaluation of Toluene in CFY Rats and CFLP Mice[a]

	Rats					Mice		
	Air Inhalation	Toluene		Air Inhalation	Toluene	Air Inhalation	Toluene	
	Days 1 to 21 8 h/d	266 ppm Days 1 to 21 8 h/d	399 ppm Days 1 to 8 24 h/d	Days 9 to 14 24 h/d	399 ppm Days 9 to 14 24 h/d	Days 6 to 13 24 h/d	133 ppm Days 6 to 13 24 h/d	399 ppm Days 6 to 13 24 h/d
No. pregnant animals examined	10	10	9	26	19	14	11	0
No. pregnant animals died	0	0	5	0	2	0	0	15
Maternal weight gain[b] (%)	46.6	44.1	44.0	46.9	41.8	--	--	
No. live fetuses	111	133	95	348	213	124	112	0
No. resorbed fetuses	8	3	6	15	18	6	10	0
No. dead fetuses	0	0	0	0	0	1	0	0
Fetal loss (%)	6.7	2.2	5.9	4.1	7.8	6.1	8.2	0
Mean litter size	11.1	13.3	10.6	13.4	11.2	9.0	10.2	--
Mean fetal weight (g)	3.8	3.6	3.3*	3.8	3.8	1.1	1.0*	--
Mean placental weight (g)	0.5	0.5	0.5	0.5	0.5	--	--	--
Weight retarded fetuses[c] (%)	7.2	16	46**	6.9	17.3	6.5	27.6**	--
External malformations[d]	0	0	0	0	0	0	0	--
No. fetuses dissected[e]	54	64	49	179	110	64	58	0
Internal malformations[f]								
Anophthalmia	0	0	0	1	0	0	0	--
Hydrocephalus	--	--	4	--	--	--	--	--
Hydronephrosis	1	6	4 *	16	4	1	3	--
No. of Alizarin-stained fetuses	57	69	42	169	102	60	54	--
Skeletal retardation signs[g]	0	17**	7**	11	24**	3	1	--

TABLE 14-8 (cont.)

	Rats					Mice		
	Air Inhalation	Toluene		Air Inhalation	Toluene	Air Inhalation	Toluene	
	Days 1 to 21 8 h/d	266 ppm Days 1 to 21 8 h/d	399 ppm Days 1 to 8 24 h/d	Days 9 to 14 24 h/d	399 ppm Days 9 to 14 24 h/d	Days 6 to 13 24 h/d	133 ppm Days 6 to 13 24 h/d	399 ppm Days 6 to 13 24 h/d
Skeletal anomalies								
Fused sternebrae	0	0	0	2	7**	0	0	--
Extra ribs	0	0	0	0	22***	0	0	--
Skeletal malformations[h]								
Missing vertebrae	0	0	0	0	2	0	0	--
Brachimelia	0	0	0	0	0	1	0	--

[a] Source: Hudak and Ungvary, 1978

[b] Percent of starting body weight

[c] Percent of living fetuses weighing <3.3 g (rats) or 0.9 g (mice)

[d] Agnathia, brachimelia, missing tail

[e] The rats were sacrificed on day 21 of pregnancy, the mice on day 18

[f] Thymus hypolasia also looked for

[g] Including poorly ossified sternebrae, bipartite vertebra centra, and shortened 13th ribs

[h] Fissura sterni and agnathia also looked for

*$P < 0.01$ (*t*-test); ** $P < 0.05$ (Mann Whitney U Test); *** $P < 0.01$ (Mann Whitney U Test)

h = hour; d = day

served as controls, and the fetuses were examined on day 21 of pregnancy. The results of the toluene exposures in this study are consistent with those of Hudak and Ungvary's continuous 399 ppm toluene exposures with rats on days 9 to 14 of gestation. Tatrai et al. (1979) concluded that continuous exposure to 266 ppm toluene was not teratogenic (no external, internal, or skeletal malformations were reported), although the exposures were associated with evidence of skeletal retardation (not detailed) and an increased incidence of extra ribs (Table 14-9). It was additionally found that the incidence of extra ribs was higher in the group exposed to toluene in combination with benzene than in the groups exposed to toluene alone. Maternal loss, maternal weight gain, number of litters, mean implantation/dam, placental weight, fetal loss, and fetal weight loss were not significantly affected by the toluene exposures. Exposure to 125 ppm benzene did cause decreases in maternal weight gain, placental weight and fetal weight, but these effects appeared to be inhibited by concurrent exposure to 266 ppm toluene. Further, it was reported that post-implantation fetal loss (the number of dead and resorbed fetuses relative to the number of total implantation sites in percent) was significantly increased in the group exposed to benzene in combination with toluene; fetal loss was not, as indicated earlier, affected by exposure to the toluene (or benzene) alone.

In a third inhalation study, Litton Bionetics, Inc. (1978b) reported no evidence of teratogenicity in the 20-day-old fetuses of Charles River rats that were exposed to 100 or 400 ppm toluene vapor for 6 hours/day on days 6 to 15 of gestation. Histological examinations revealed no unusual incidence of visceral or skeletal abnormalities (Table 14-10); unusual skeletal variations were observed in a small but comparable number of fetuses from both the exposed and control groups, but these changes were in most cases attributed to retarded bone ossification and were not considered to be malformations as such. It was also noted that there were no maternal deaths during this study, and that the sex ratio of the offspring did not differ significantly between the treated and control groups.

In a brief abstract, Roche and Hine (1968) noted that toluene was not teratogenic to either the rat fetus or the chick embryo. Parameters evaluated included body weight, bone length, and gross abnormalities, but no dose or exposure information or other quantitative data were provided.

Elovaara et al. (1979) injected toluene into the air space of developing chicken eggs at doses of 5, 25, 50, and 100 μmol/egg on the 2nd and 6th days of

TABLE 14-9

Teratogenic Effects of Exposure to Toluene, Benzene, and a Combination of Toluene and Benzene in CFY Rats[a]

Inhalation on days 7 to 14 of pregnancy 24 h/d	Air	Toluene 266 ppm	Benzene 125 ppm	Toluene/Benzene 266 ppm + 125 ppm	Significance of Interaction
Number of females					
treated	21	20	20	20	
died	--	--	--	--	--
non pregnant	1	2	3	1	
total resorption	--	--	--	--	
Number of liters	21	18	17	19	
Mean implantation/dam	14.0	14.4	14.6	13.8	
Maternal weight gain in % of starting body weight	68.82 ±2.40	65.82 ±2.13	46.74*** ±2.69	53.94*** ±1.84	$p < 0.05$
Relative liver weight (%)	4.25 ±0.08	4.37* ±0.07	4.67* ±0.12	4.10 ±0.09	$p < 0.01$
Mean placental weight (g)	0.58 ±0.006	0.60 ±0.006	0.48*** ±0.006	0.54*** ±0.004	$p < 0.05$
Number of fetuses	294	259	248	262	
live	280	239	236	234	
dead	--	--	2	--	
resorbed	14	20	10	28	
Mean fetal weight (g)	3.94 ±0.02	3.91 ±0.02	3.16*** ±0.03	3.79** ±0.02	$p < 0.001$
Weight retarded fetuses in % of living fetuses	2.8	3.3	57.6**	9.8*	
External malformations	--	--	--	--	
Fetal loss/total implantation sites (%)	4.7	7.7	4.8	10.7*	
No. Alizarine-stained fetuses	142	121	122	118	
Skeletal retarded fetuses in % of Alizarine-stained fetuses	13	31*	77***	39*	

TABLE 14-9 (cont.)

Inhalation on days 7 to 14 of pregnancy 24 h/d	Air	Toluene 266 ppm	Benzene 125 ppm	Toluene/Benzene 266 ppm + 125 ppm	Significance of Interaction
Skeletal anomalies					
sternum misaligned	4	4	5	1	
asymmetric vertebra	1	--	3	1	
extra ribs	1	7^+	1	19**	
Skeletal malformations	--	--	--	--	
No. fetuses dissected	138	118	114	116	
Internal malformations					
polycystic lungs	1	--	--	--	
pyelectasia	2	5	--	1	
dystopia renis	--	1	--	--	
vesica giganta	--	3	1	1	
microphthalmia	--	--	--	1	
anophthalmia	--	--	2	--	
hydrocephalus internus	--	--	3	--	

[a] Source: Tatrai et al., 1980

$^+$ = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ± = SEM

TABLE 14-10

Teratogenicity and Reproductive Performance Evaluation in Rats Exposed to Toluene[a]

	Dose (ppm)		
	0	100	400
Pregnancy ratio (Pregnant/Bred)	26/27	27/27	27/27
No. pregnant rats that died	0	0	0
Live litters	26	27	26
Implantation sites (Left Horn/Right Horn)	152/194	181/177	179/190
Resorptions	26	28	41[b]
Litters with resorptions	13	20	17
Dead fetuses	0	1	0
Litters with dead fetuses	0	1	0
Live fetuses/implantation site	320/346	329/358	328/369
Mean live litter size (fetuses)	12	12	12
Average fetal weight (g)	3.6	3.5	3.8
Number of fetuses examine for soft tissue (visceral) changes[c]	108(51/57)	105(47/58)	104(51/53)
Number of fetuses examined for skeletal changes[d]	212	221	224
Number of fetuses with normal skeletal examinations	139	150	158
Fetuses with commonly encountered skeletal changes[e,f]	67(20)	62(20)	58(20)
Fetuses with unusual skeletal variations[f,g]	6(4)	9(4)	8(6)

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[a] Source: Litton Bionetics, Inc., 1978b

[b] The increase in total resorptions at this dose was attributed to the total resorption of the litter of one particular female.

[c] Numbers of male/females examined in parentheses.

[d] Four specimens from one litter were not examined (missing).

[e] A qualitative examination of the observations recorded for the fetuses indicates that bilateral ribs, unilateral ribs, and reduced ossification of various bones were the most frequently encountered changes.

[f] Number of litters in parenthesis.

[g] These were generally cases of more severe and extensive retarded ossification.

incubation. Survival incidence after 14 days of incubation appeared to be influenced only after injection of toluene on day 6 at 100 μmol/egg; the "approximate LD_{50}" for toluene was judged to be in excess of 100 μmol/egg. Macroscopic examination on day 14 indicated that only 3 of 46 of the chick embryos treated with 5 to 100 μmol/egg of toluene were malformed; 1 displayed profound edema and 3 had skeletal abnormalities (musculoskeletal defects of the lower extremities, but not wings).

McLaughlin et al. (1964) injected toluene at dose levels of 4.3, 8.7, and 17.4 mg into the yolk sac of fresh fertile chicken eggs before incubation. Following incubation, the percentages of hatch at the 3 doses were, respectively, 85%, 25%, and 0%. Teratogenic effects were not observed in either the eggs that failed to hatch or in the chicks that did hatch.

14.3.2. Human Reports. Holmberg (1979) gathered information on exposure to noxious agents during the pregnancies of 120 mothers of children with congenital CNS defects and their matched-pair controls. The matched-control mother is the mother whose delivery immediately preceded that of the case mother in the same Finnish maternity welfare district. Results showed that 14 of the 120 case mothers had been exposed more often than control mothers (3/120) to organic solvents during the first trimester of pregnancy. Among the 14 exposed mothers, 2 had been exposed to toluene. One of the toluene-exposed mothers (age 18) had reportedly been exposed in the metal products manufacturing industry (no other details of exposure given), and gave birth to a child that died after 2 hours and showed internal congenital hydrocephaly and agenesis of the corpus callosum upon autopsy; other findings included pulmonary hypoplasia and a diaphragmatic hernia. The other mother was exposed to toluene concomitantly with other solvents (xylene, white spirit, methyl ethyl ketone) during rubber products manufacturing; her child was hydranencephalic and died 24 days after birth. It was noted that in this case parental age (maternal, 42 years; paternal, 44 years) and a previous child with brain injury (born 20 years previously, died at age 4) were more likely than the recent exposure to have predisposed the more recent child to the defect.

Toutant and Lippman (1979) described the birth of a child with "nearly classic" fetal alcohol syndrome to a 20-year-old primigravida whose major addiction was to solvents (reportedly, primarily toluene). This woman had a 14-year history of daily heavy solvent abuse (no details provided) and a 3-year history of alcohol intake of about a six-pack of beer weekly. On admission, she exhibited signs compatible with severe solvent and/or alcohol abuse (ataxia, resting and intention tremors, mild diffuse sensory deficits, short-term memory loss, and poor intellectual functioning). The child was born at term, was small (10th percentile in weight, 5th percentile in head size), and exhibited abnormal features that included microcephaly, a flat nasal bridge, hypoplastic mandible, short palpebral fissures, mildly low-set ears, pronounced sacral dimple, sloping forehead, and uncoordinated arm movements. It was noted that although solvent abuse rather than alcohol predominated in this mother's addiction pattern, the case seemed no different from reports of fetal alcohol syndrome.

14.4. SUMMARY

CIIT (1980) concluded that exposure to 30, 100, or 300 ppm toluene for 24 months did not produce an increased incidence of neoplastic, proliferative, inflammatory, or degenerative lesions in rats relative to unexposed controls; the highest dose tested was not, however, a maximum tolerated dose. Other studies indicate that toluene is not carcinogenic when applied topically to the shaved skin of mice (Pohl, 1973; Lijinsky and Garcia, 1972; Coombs et al., 1973; Doak et al., 1976), and that it does not promote the development of epidermal tumors following initiation with DMBA (Frei and Kingsley, 1968; Frei and Stephens, 1968).

Toluene has yielded negative results in a battery of microbial, mammalian cell, and whole organism test systems. The microbial assays conducted include differential toxicity testing with wild-type and DNA repair-deficient strains of *E. coli* and *S. typhimurium* (Fluck et al., 1976; Mortelmans and Riccio, 1980), reverse mutation testing with various strains of *S. typhimurium*, *E. coli* WP2, and *S. cerevisiae* D7 (Litton Bionetics, Inc., 1978a; Mortelmans and Riccio, 1980; Nestman et al., 1980), and mitotic gene conversion and crossing-over evaluation in *S. cerevisiae* D4 and D7 (Litton Bionetics, Inc., 1978a; Mortelmans and Riccio, 1980). Toluene also failed to induce specific locus forward mutation in the L5178Y Thymidine Kinase mouse lymphoma cell assay (Litton Bionetics, Inc., 1978a), was negative in the micronucleus test in mice (Kirkhart, 1980), and was negative in the mouse dominant lethal assay (Litton Bionetics, Inc., 1981). Sister-chromatid exchange (SCE) frequencies were not altered in Chinese hamster ovary cells (Evans and Mitchell, 1980) or in human lymphocytes (Gerner-Smidt and Friedrich, 1978) cultured with toluene *in vitro*. The frequency of SCEs in peripheral lymphocytes from workers that had a history of chronic exposure to similar levels of toluene has been reported to be increased (Bauchinger et al.,

1982) as well as unchanged (Funes-Craviota et al., 1977; Maki-Paakkanen et al., 1980).

In the Russian literature, chromosome aberrations were reported in the bone marrow cells of rats exposed subcutaneously (Dobrokhotov, 1972; Lyapkalo, 1973) and via inhalation (Dobrokhotov and Enikeev, 1977) to toluene. These findings were not corroborated, however, in a Litton Bionetics, Inc. (1978b) study in rats following intraperitoneal injection or in cultured human lymphocytes exposed to toluene in vitro (Gerner-Smidt and Friedrich, 1978). An excess of chromosome aberrations in lymphocytes from workers who were chronically exposed to similar levels of toluene has been reported by Bauchinger et al., 1980 and Funes-Craviota et al., 1977, but not by Forni et al., 1971 or Maki-Paakanen et al., 1980; it is probable, however, that part of the exposure in the Funes-Craviota et al. (1977) study was to benzene-contaminated toluene.

Toluene was reported in a recent abstract from NIEHS to induce cleft palates at a level of 1.0 mℓ/kg (0.87 g/kg) following oral exposure to mice on days 6 to 15 of gestation (Nawrot and Staples, 1979); significant increases in embryolethality and decreases in fetal weight were noted as well at doses as low as 0.3 m/kg/day and 0.5 m/kg/day, respectively. The teratogenic effect reportedly did not appear to be due merely to the general retardation in growth rate. Three other studies concluded that toluene is not teratogenic in mice (Hudak and Ungvary, 1978) or rats (Hudak and Ungvary, 1978; Litton Bionetics, Inc., 1978b; Tatrai et al., 1980) following inhalation exposure. Embryotoxic effects (increased incidence of skeletal anomalies and signs of retarded skeletal development, low fetal weights) and increased maternal mortality were noted, however, in some of the rats and mice exposed via inhalation. Injection of toluene into the yolk sac (McLaughlin et al., 1964) or air space (Elovaara et al., 1979b) of chicken eggs before incubation or during development, respectively, did not result in teratogenic effects.

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15. SYNERGISMS AND ANTAGONISMS AT THE PHYSIOLOGICAL LEVEL

15.1. BENZENE AND TOLUENE

Animal studies have shown that benzene and toluene may be metabolized by similar enzyme systems in the parenchymal cells of the liver. Pawar et al. (1976) found that the activities of hepatic aminopyrine N-demethylase, NADPH-linked peroxidation, and ascorbate-induced lipid peroxidation were reduced, while acetanilide hydroxylase was increased, by either benzene pretreatment or toluene pretreatment in male rats. Induction of aminopyrine N-demethylase and components of the electron transport system was seen when the animals were given phenobarbital (Pawar et al., 1976; Mungikar and Pawar, 1976). When phenobarbital was coadministered with benzene or toluene, the changes in the activity of these enzymes produced by single administration of the xenobiotics were attenuated (Pawar et al., 1976). That induction of hepatic enzymes by phenobarbital affects metabolism of toluene is indicated by the reduction of toluene toxicity (decreased narcosis) in female rats or male mice given phenobarbital prior to intraperitoneal injection of toluene (Ikeda and Ohtsuji, 1971; Koga and Ohmiya, 1978), and the accelerated excretion of toluene metabolites from female rats as described in Sections 12.3. and 12.4. (Ikeda and Ohtsuji, 1971).

The following studies indicate that toluene has the potential for altering the bioactivity of benzene when given in sufficiently large quantities. When benzene was given in combination with toluene, the conversion of benzene to its metabolites (phenols) was suppressed in rats (Ikeda et al., 1972) and in mice (Andrews et al., 1977). Ikeda et al. (1972) administered a mixture of benzene and toluene (equivalent to 110 mg benzene/kg and 430 mg toluene/kg) intraperitoneally to female rats and observed a reduced excretion of total phenols. When a mixture of toluene and benzene (110 mg toluene/kg and 440 mg benzene/kg) was administered, hippuric acid excretion was reduced up to 4 hours after injection. Induction of hepatic microsomal enzymes by phenobarbital prior to administration of the mixture alleviated the suppression.

Andrews et al. (1977) co-administered 440 or 880 mg/kg benzene and 1720 mg/kg toluene intraperitoneally to mice and found a significant reduction in urinary excretion of benzene metabolites and a compensatory increase of pulmonary excretion of unmetabolized benzene. When toluene and benzene were coadministered by subcutaneous injection, toluene did not significantly change the total amount of benzene found in fat, liver, spleen, blood, or bone marrow, but it did reduce significantly the accumulation of metabolites in these tissues. Coadministration of toluene and benzene also counteracted benzene-induced reduction of red cell ^{59}Fe uptake in developing erythrocytes, suggesting that the myelotoxicity of benzene might be attenuated by toluene-inhibition of benzene metabolism in the bone marrow. In an in vitro study with a liver microsome preparation, Andrews and coworkers (1977) determined that toluene is a competitive inhibitor of benzene metabolism.

In the studies of Ikeda et al. (1972) and Andrews et al. (1977), benzene and toluene were administered intraperitoneally in large amounts. Sato and Nakajima (1979), however, used doses in the range of 24.2 to 390.6 mg/kg of benzene and 28.6 to 460.8 mg/kg of toluene to assess the effects of concentrations that might be found in the workplace. They found that when benzene was given to rats in the range of 24.2 to 97.7 mg/kg, there was no significant difference in the rate of disappearance of benzene from the blood whether the benzene was administered singly or in combination with an equimolar amount of toluene. At a dose of 390.6 mg/kg benzene, an equimolar dose of toluene delayed the disappearance of benzene from blood, and the excretion of phenol was reduced. A dose-dependent inhibition of the metabolism of benzene by toluene was found. In a study of human exposure, inhalation of a mixture of 25 ppm benzene and 100 ppm toluene for 2 hours did not exert any influence on the disappearance rate of benzene and toluene in either blood or end-tidal (alveolar) air when compared to inhalation of either solvent singly. Desaturation curves (concentration versus time) for blood or end-tidal air for each solvent after inhalation of the specified mixture were virtually identical to desaturation curves obtained after inhalation of the same solvent (25 ppm benzene or 100 ppm toluene) by itself. These results indicate that in the range of threshold limit value "the pharmacokinetic processes . . . of absorption, distribution, excretion, and metabolism of either benzene or toluene are not influenced by simultaneous exposure to the other" (Sato and Nakajima, 1979). The data for the single-solvent exposures had been published previously (Sato et al., 1974); details of the experiment with toluene were discussed in Section 12.4.

15.2. XYLENES AND TOLUENE

When 0.1 mℓ/kg or 0.2 mℓ/kg toluene was co-administered with similar doses of m-xylene intraperitoneally into male rats, the amounts of hippuric and m-methylhippuric acid excreted in urine over a period of 24 hours were not different from the amount of metabolites formed by single injections of toluene or m-xylene. The velocity of excretion of metabolites in the simultaneously injected group was slightly slower than that in the singly injected groups. Thus, simultaneous administration of the compounds does not significantly interfere with the metabolism of either compound (Ogata and Fujii, 1979).

To study the excretion kinetic interactions between toluene and xylene, Riihimaki (1979) determined the conjugation and urinary excretion of metabolites of toluene m-xylene, benzoic acid and methylbenzoic acid in vivo in one adult human male. Forty-one mmol benzoic acid or 7.4 mmol methylbenzoic acid was ingested singly or in combination by the subject. Urine was collected for 25 to 30 hours after ingestion; the total recovery of the ingested compounds with the exception of one sample (84% of the dose excreted in that case) indicated that all elimination took place via the kidneys. Combined intake of methylbenzoic acid and benzoic acid did not significantly affect conjugation or excretion of either metabolite. This study indicates that during simultaneous exposures to toluene and m-xylene, even at relatively high occupational exposure levels, conjugation and excretion of metabolites are not likely to be rate-limiting steps except under conditions of limited availability of glycine.

15.3. TOLUENE AND OTHER SOLVENTS

Simultaneous intraperitoneal injection of 1.18 g/kg toluene and 0.91 g/kg n-hexane into female rats did not affect the concentrations of n-hexane in the blood nor excretion of hippuric acid (Suzuki et al., 1974).

Impaired peripheral (tail) nerve function (as indicated by decreased motor nerve conduction velocity and mixed nerve conduction velocity, and increased distal latency) was observed in rats after 8, 12, and 16 weeks' exposure to 1000 ppm n-hexane for 12 hours/day (Takeuchi et al., 1981). Similar exposure to a mixture of 1000 ppm n-hexane plus 1000 ppm toluene resulted in only slight impairment, and exposure to 1000 ppm toluene alone had a negligible effect on the above indices. Clinical signs of neuropathy were not observed in any of the groups throughout the experiment.

Coadministration of ethanol by ingestion and toluene by inhalation (1060 ppm, 6 hours daily, 5 days a week for 4 weeks) to rats did not change the electrocardiogram, hematocrit values, or histological and histochemical structure of the heart. Toluene increased vascular resistance of the myocardium and reduced cerebral blood flow, while alcohol ingestion reduced arterial blood pressure, the cardiac index, and blood flow to the myocardium, kidney, skin, and carcass. Myocardial and cutaneous vascular resistance, as well as cerebral blood flow, increased after alcohol ingestion. It was concluded that combined exposure to the two substances produced additive effects on myocardial vascular resistance (Morvai and Ungvary, 1979). During subchronic exposure of rats to toluene and ethanol, there is a potentiation of microsomal and mitochondrial changes in the liver (Hudak et al., 1978). Ethanol administered to rats in single oral doses of 4 g/kg enhanced the *in vitro* metabolism of toluene without causing an increase in the microsomal protein and cytochrome P-450 contents (Sato et al., 1981). The enhancement was greatest (about twofold) at the time when ethanol was disappearing from the body, i.e., 16 to 18 hours after ethanol administration.

Smyth et al. (1969) suggested in a study of joint toxic action that perchloroethylene is capable of enhancing the toxicity of orally administered toluene in rats. Withey and Hall (1975) observed that intubation administration of trichloroethylene and toluene to rats, in combinations of mixtures at five different dose levels, revealed a departure from an additive model. They concluded that the effect of co-administration of the solvents could not be described in terms of synergism or potentiation until further studies were made.

Ikeda (1974) found that coadministration of trichloroethylene and toluene (730 mg/kg and 430 mg/kg, respectively) to rats by the intraperitoneal route reduced the amounts of metabolites of both solvents compared with amounts excreted after administration of either solvent alone.

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16. ECOSYSTEM CONSIDERATIONS

16.1. EFFECTS ON VEGETATION

16.1.1. Introduction. Toluene volatilizes rapidly from solutions (Mackay and Wolkoff, 1973). Most studies investigating the phototoxicity of toluene have been with algae. Of these studies, only one (Dunstan et al., 1975) was done under conditions that maintained a nearly constant concentration of toluene in the culture medium throughout the experiment. Other studies were done with culture vessels capped with metal caps or with cotton plugs, allowing the toluene to volatilize and escape from the exposure solutions. Even though steady-state concentrations are lacking, these studies do approximate situations in the environment where a point source of toluene exists to a body of water. The discussion of these studies will, therefore, be under the headings of "closed" and "open" experimental systems.

16.1.2. Effects of Toluene on Plants.

16.1.2.1. ALGAE

16.1.2.1.1. Closed System Studies -- Dunstan et al. (1975) exposed 4 marine algal species to toluene concentrations ranging from 1 to 10^5 µg/ℓ. Axenic algal cultures were inoculated at 18°C and grown with a 12-hour light/dark cycle under cool-white fluorescent light (4000 µW/cm^2, 380 to 700 nm) in filtered enriched seawater. To minimize loss of toluene by vaporization, the 125 mℓ Erlenmeyer flasks were made airtight with rubber stoppers. Experiments were never run beyond a cell density at which CO_2 limitations might limit growth. The four species used were the diatom, *Skeletonema costatum*; the dinoflagellate, *Amphidinium carterae*; the cocolithophorid, *Cricosphaera carterae*; and the green flagellate, *Dunaliella tertiolecta*.

To illustrate the difficulty of establishing absolute concentration when working with toluene, Dunstan et al. (1975) observed the toluene concentrations at three intervals in stoppered flasks (Table 16-1). Eighty-four percent of the theoretical initial concentration was lost at the beginning of the experiment during the handling and dispensing of the toluene into culture flasks, even when the toluene was rapidly dispensed under sterile conditions.

Figure 16-1 shows how toluene can both stimulate and inhibit algal growth depending on the species and the concentration of toluene. The dinoflagellate, *Amphidinium carterae*, was inhibited at all concentrations of toluene (1 to

TABLE 16-1

Concentrations of Toluene in Stoppered Flasks[a]

Time of Measurement	Percent of Theoretical Concentration
Theoretical initial concentration	100
Measured initial concentration	16
Concentration after 3 days of growth	
Stoppered flask	14
Cotton-plugged flask	1

[a] Source: Dunstan et al., 1975

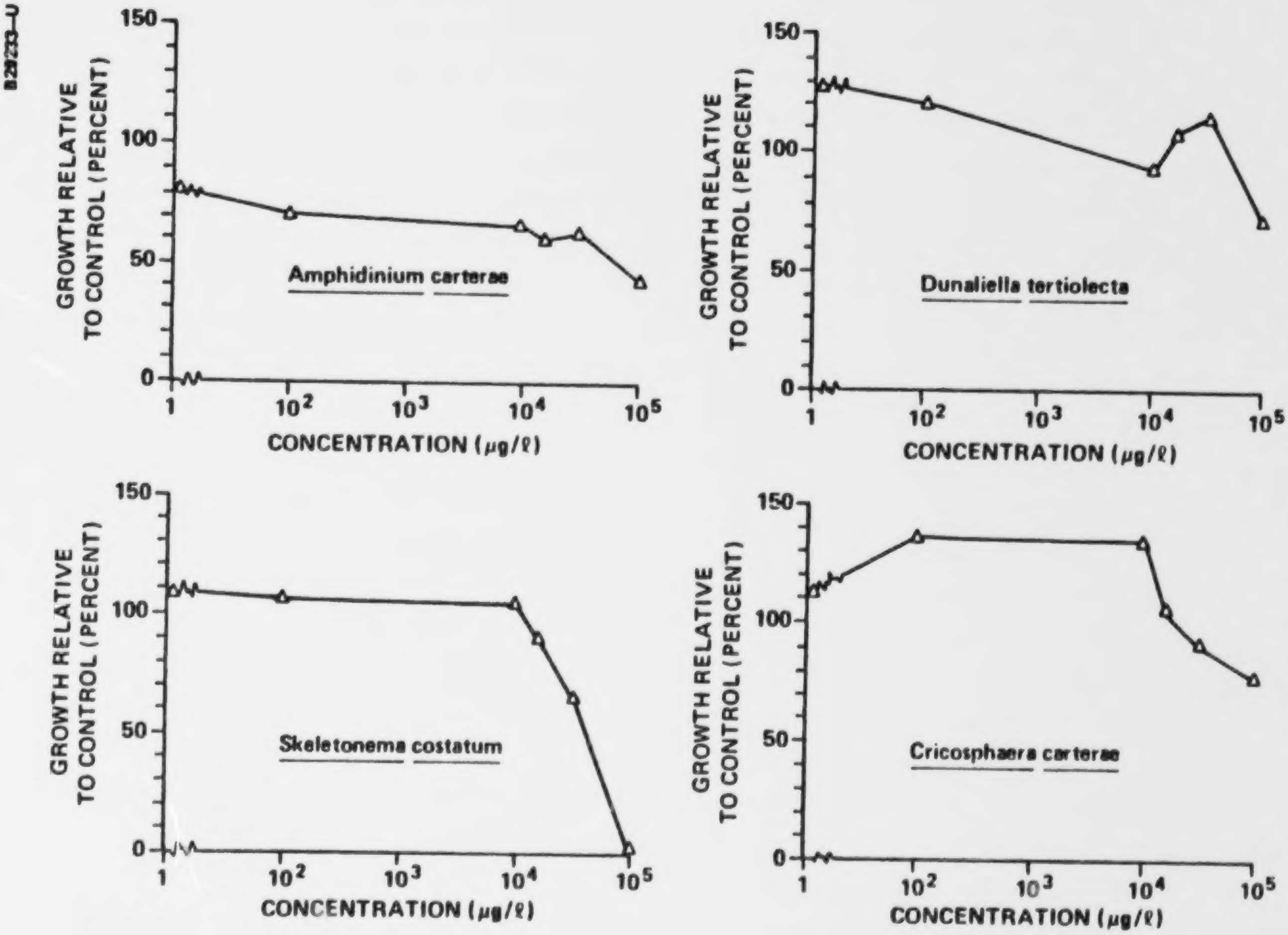

FIGURE 16-1

Phytoplankton Growth in Various Concentrations of Toluene (Organisms were grown in stoppered flasks. Growth, measured by cell numbers and *in vivo* chlorophyll, was determined on the 2nd and 3rd days of logarithmic growth. Concentrations of low molecular weight hydrocarbons are in theoretical values.)

Source: Dunstan et al., 1975

10^5 μg/ℓ) from 20 to 50%. The other three species, however, were stimulated by 1 to 10^4 μg/ℓ, but higher concentrations of toluene either had no effect (*Dunaliella tertiolecta*) or became inhibitory (*Skeletonema costatum* and *Cricosphaera carterae*). This work indicated that one of the most significant environmental effects was in the short-term selection of certain phytoplanktonic species by the growth stimulation brought about by low levels of toluene. Dunstan et al. (1975) concluded that the differential growth of phytoplanktonic species within the phytoplankton population ultimately determines the community structure, its succession, and its trophic relationship.

Potera (1975) evaluated the effect of toluene on saltwater phytoplankton dominated by *Chlorella* sp. using Warburg manometry. Toluene inhibited photosynthesis 29% at 34 mg/ℓ and 35% at 342 mg/ℓ (at 20≈C). Respiration (at 20≈C) was inhibited 62% at 34 mg/ℓ and 16% at 342 mg/ℓ.

16.1.2.1.2. Open Studies -- Illustrative of the "open" type of experiment is that of Kauss and Hutchinson (1975). The freshwater alga, *Chlorella vulgaris*, was exposed to toluene for 10 days in 125 mℓ cotton-plugged Erlenmeyer flasks. Each flask was agitated to resuspend the cells daily. The concentrations listed in Figure 16-2 are nominal initial concentrations. In this open experiment, toluene was less toxic to the alga because the toluene concentration diminished by volatilization during the experiments. Comparison with controls revealed that a lag phase that lasted for 1 day existed between inoculation and commencement of growth for 50 and 100 mg/ℓ. Recovery was less rapid with 250 mg/ℓ. At concentrations approaching toluene saturation (i.e., 505 mg/ℓ), toluene was lethal to the cells.

Table 16-2 summarizes the toxic effects of toluene on algae. In assessing the toxicity of toluene to algae, both the inherent toxicity of toluene and the exposure time need to be considered. The no-effect concentration for most algal species studied appears to be at the 10 mg/ℓ level. The evaporation rate from solution (fresh or saltwater), however, rapidly diminishes the exposure concentration of toluene (Dunstan et al., 1975). The toxicity of toluene is more closely approximated by levels of 100 mg/ℓ in "open" systems, as shown by Kauss and Hutchinson (1975).

16.1.2.2. EFFECTS ON HIGHER PLANTS -- Currier (1951) exposed barley, tomatoes, and carrots to toluene vapor. Air at a flow rate of 11.5 ℓ/min passed through a small vaporizing chamber containing the toluene and into the top of a bell jar containing the plants. The concentration of toluene in the vapor

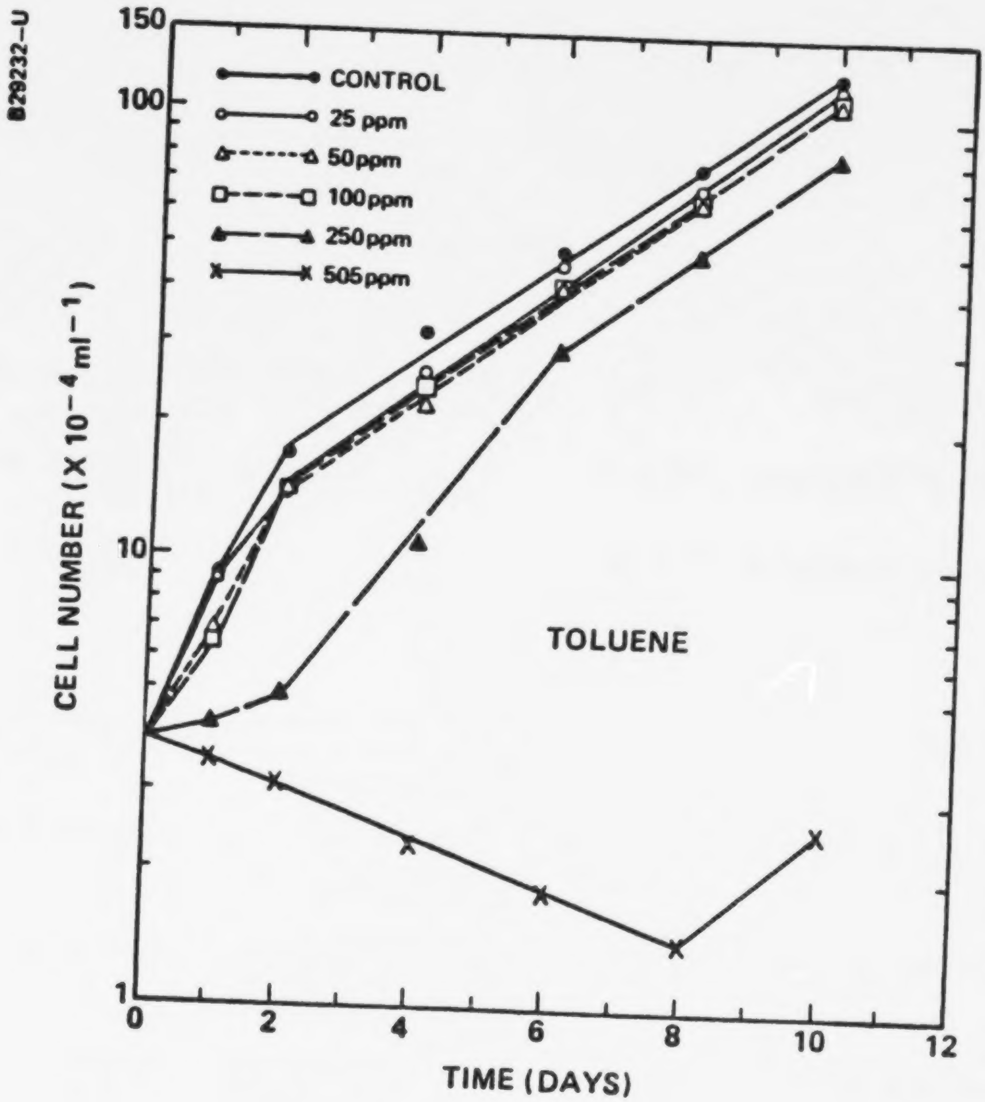

FIGURE 16-2

Growth of Chlorella vulgaris in Medium Containing Toluene (Data plotted are the average of three replicates. Lines of best fit were determined using regression coefficients. Numbers represent initial hydrocarbon concentration on a parts per million basis. The arrow on the ordinate indicates starting cell concentration.)

Source: Kauss and Hutchinson, 1975

TABLE 16-2

Toxic Effects of Toluene to Algae

Species	Concentration	Effect	Reference
FRESHWATER			
Chlorella vulgaris	245 mg/ℓ	24 h EC_{50} (cell number)	Kauss and Hutchinson, 1975
Chlorella vulgaris	250 mg/ℓ	96 h no-effect conc. (cell number)	Kauss and Hutchinson, 1975
Microcystis aeruginosa	105 mg/ℓ	8 d no-effect conc. (chlorophyll *a*)	Bringmann and Kuhn, 1978
Scenedesmus quadricauda	>400 mg/ℓ	8 d no-effect conc. (chlorophyll *a*)	Bringmann and Kuhn, 1978
SALTWATER			
Amphidinium carterae	<0.001 mg/ℓ	2 to 3 d no-effect conc. (cell number and chlorophyll)	Dunstan et al., 1975
Dunaliella tertiolecta	10 mg/ℓ	2 to 3 d no-effect conc. (cell number and chlorophyll)	Dunstan et al., 1975
Skeletonema costatum	10 mg/ℓ	2 to 3 d no-effect conc. (cell number and chlorophyll)	Dunstan et al., 1975
Cricosphaera carterae	10 mg/ℓ	2 to 3 d no-effect conc. (cell number and chlorophyll)	Dunstan et al., 1975
Ectocarpus sp.	1730 mg/ℓ	inhibits asexual spore germination	Skinner, 1972
Enteromorpha sp.	1730 mg/ℓ	inhibits asexual spore germination	Skinner, 1972

h = hour; conc. = concentration; d = day.

chamber was varied by changing the temperature of the toluene. The concentration of vapor in the air was determined by measuring the amount of toluene evaporated per unit of time. Three tomatoes, 20 carrots, and 12 barley seedlings were tested 32, 32, and 14 days respectively after planting. Plants were exposed in the gas chamber for 1/4, 1/2, 1, and 2 hours. The type and extent of injury were recorded after 1 month to allow for a recovery period. Temperature of the plants was held at 25°C.

Results showed that toxic effects of toluene vapor were influenced by exposure period and dosage (Table 16-3). Toluene was observed to be toxic at concentrations of 6.4 to 12.0 mg/ℓ after 15 minutes of exposure (Currier, 1951). Fifteen minutes of exposure at 12 mg/ℓ toluene produced a 50, 0, and 60% injury to tomato, carrot, and barley, respectively. The effects of the exposures on flower and fruit development were not determined. For lethality to occur at 12.0 mg/ℓ, barley required 1 hour, tomato 2 hours, and carrot over 2 hours. The toxicity appeared to vary markedly within a narrow limit. By lowering the concentration of toluene from 12.0 to 6.4 mg/ℓ, the percentage of injury to barley after a 2-hour exposure was reduced from 100% (lethal) to 15%. At 24.1 mg/ℓ, toluene was only twice as toxic to barley seedlings as at 12.0 mg/ℓ after a 30-minute exposure.

Toluene entered the plant rapidly through the cuticle and stomata. Symptoms of injury included a darkening of the tips of the youngest leaves, presumably as a result of leakage of sap into the cellular spaces (Currier, 1951). This darkening spread to the older leaves. There was a loss of turgor, with draping stems and leaves. In bright sunlight, the chlorophyll was destroyed.

Toluene is classified as a contact poison that quickly kills the plant tissue with which it comes in contact (Currier, 1951). This material is not accumulated in plants nor is it translocated. The mechanism of toxicity involves disorganization of the outer membrane of the cell due to solvent action on the lipoid constituents, resulting in disruption of photosynthesis, respiration, and turgor pressure.

16.2. BIOCONCENTRATION, BIOACCUMULATION, AND BIOMAGNIFICATION POTENTIAL

Limited information is available concerning toluene's potential for accumulating in aquatic organisms and aquatic food chains. Possible pathways of toluene uptake are directly from water (bioconcentration) and from both water and food (bioaccumulation). Biomagnification occurs if the concentration of a com-

TABLE 16-3

Toxic Effects of Toluene Vapor on Carrots, Tomatoes, and Barley[a]

Material	Concentration	Percent Injury[b]			
		Exposure Time (h)			
		1/4	1/2	1	2
Tomato	12.0 mg/ℓ	50	60	75	100
Carrot	12.0 mg/ℓ	0	50	75	75
Barley	12.0 mg/ℓ	60	50	98	100
Barley	6.4 mg/ℓ	0	25	15	15
Barley	24.1 mg/ℓ	ND	100	100	ND

[a] Source: Currier, 1951

[b] 0% = no effect; 100% = lethal 1 month after exposure.

h = hour; ND = not determined.

pound in an organism increases with its trophic level as a result of passage through food chains.

Nunes and Benville (1979) studied the uptake and depuration of toluene and other monocyclic aromatic components of the water-soluble fraction (WSF) of Alaskan Cook Inlet crude oil in Manila clams (*Tapes semidecussata*). Clams were exposed for 8 days to a constant WSF concentration under continuous-flow exposure conditions. The toluene concentration in water was measured daily. The toluene concentration in a pooled sample of 10 clams was measured at 2, 4, 6, and 8 days. At the end of the exposure period, remaining clams were transferred to clean-flowing seawater and pooled tissue samples were analyzed for toluene after 1, 7, and 12 days of depuration. The data are provided in the following tabulation:

	Days	Toluene Concentration (ppm)	
		Water	Tissue
Exposure			
	1	1.2	--
	2	1.3	2.3
	3	1.7	--
	4	1.4	2.2
	5	1.2	--
	6	0.9	0.87
	7	1.0	--
	8	1.1	2.0
Depuration			
	1	--	3.30
	7	--	0.80
	14	--	1.10

The mean water concentration during the uptake period was 1.2 ppm toluene. Tissue concentrations reached a maximum by 2 days of exposure and remained relatively constant except for a temporary decline on day six. The average tissue concentration during the exposure period was 1.5 ppm. The calculated bioconcentration factor (BCF) is 1.25 (which is equivalent to 1.5 ppm in tissue and 1.2 ppm in water). The depuration study showed that toluene was lost rapidly during the first week of depuration, but that a significant concentration of toluene remained in the clams by 2 weeks after beginning depuration.

Hansen et al. (1978) investigated the uptake and depuration of ^{14}C-toluene by blue mussels (*Mytilus edulis*). Groups of mussels were exposed under static conditions to four concentrations of ^{14}C-toluene for up to 8 hours, followed by exposure to clean, recirculating seawater for up to 192 hours. The ^{14}C-toluene concentration in water and tissue (pooled sample from four mussels) was measured by liquid scintillation counting at 1, 2, 4, and 8 hours after beginning the uptake phase and periodically in tissue during the depuration phase.

The ^{14}C-toluene concentration in tissue exceeded the water concentration by 1 hour at all exposure concentrations except the highest (40 μℓ/kg = ppm), which was toxic as shown by closure of the mussels at this concentration (Hansen et al., 1978). Equilibrium was reached by 4 hours in all groups. The BCF values at 8 hours, expressed as the tissue concentration divided by the mean water concentration, were as follows:

Water concentration (μℓ/kg)	BCF
0.05	3.8
0.4	5.7
4.0	3.6
4.0	3.6

The BCF values, which averaged 4.2, seemed to be independent of the exposure concentration, indicating that accumulation was proportional to the level in water (Hansen et al., 1978). More than half of the accumulated ^{14}C-toluene was eliminated by 1 hour after the depuration phase began at all exposure concentrations. The depuration time by which no ^{14}C-toluene was detectable in tissue was 1 hour in the mussels exposed to 0.05 μℓ ^{14}C-toluene/kg, 4 hours for those exposed to 0.4 μℓ/kg, 120 hours for those exposed to 4 μℓ/kg, and 192 hours for the animals exposed to 40 μℓ/kg.

Lee et al. (1972) reported that the same species of mussel (Mytilus edulis) took up 3 to 10 μg of ^{14}C-toluene per mussel (average dry weight tissue = 0.3 g) during static exposure for an unspecified period of time to 0.1 to 0.5 mg/ℓ. Using tissue toluene concentrations of 10 to 33 μg/g, the BCF is calculated to have been between 66 and 100. Because these values are based on dry tissue weights rather than wet weight, they are considerably higher than those reported by Nunes and Benville (1979) and Hansen et al. (1978).

Berry (1980) investigated the uptake of ^{14}C-toluene by bluegill sunfish (Lepomis macrochirus) and crayfish (Orconectes rusticus). The exposure solutions were prepared by adding 1 mℓ of ^{14}C-toluene to 100 ℓ of water for the fish experiment and by adding 1 mℓ ^{14}C-toluene to 10 ℓ of water for the crayfish experiment. A group of 40 animals was added after thorough mixing of the solutions. Duplicate water samples and 2 to 4 animals were taken at 0, 0.5, 1, 2, 4, 8, 12, 16, 20, 24, and 48 hours after beginning exposure. The ^{14}C-toluene concentration, expressed as nanograms per milligram (= ppm), was determined in water and in 7 (crayfish) or 9 (fish) tissues or organs by liquid scintillation counting. The BCF for each tissue was also calculated. Analysis of water samples showed that the toluene concentration in water decreased at a much greater rate in the crayfish experiment than in the bluegill experiment (89% versus 51% loss by 48 hours). The maximum BCF of bluegill tissues ranged from about 3 for brain to 45 for spleen. Fish muscle tissue was not analyzed. The maximum BCF for most fish tissues was reached by 8 hours. The maximum BCF of crayfish tissues ranged from about 8 for muscle to 140 for hepatopancreas. The BCF values increased throughout the 48-hour exposure period for all tissues except testes and muscle. These results indicate that toluene is accumulated above the water concentration by many tissues in these two species. The BCF of eight in the edible portion (muscle) of crayfish is considered to be a minimum value because of the rapidly decreasing toluene exposure concentration during this experiment.

Berry et al. (1978) also measured the uptake of ^{3}H-toluene by fed and unfed mosquito (Aedes aegypti) larvae and the uptake of ^{3}H-toluene by fed larvae in the presence or absence of benzene. The larvae were exposed to an initial concentration of 0.5 mℓ ^{3}H-toluene/ℓ water. Duplicate water samples and 2 to 5 larvae were taken at 1, 2, 4, 8, 12, 16, 20, and 24 hours and counted individually by liquid scintillation counting. Maximum ^{3}H-toluene counts per minute (cpm) were equal in fed and unfed larvae, but were reached more quickly (1 hour versus 4 hours) by the fed animals. The ^{3}H-toluene counts per minute values in larvae, expressed as the percentage of initial water counts, were greater during the first 4 hours in the benzene and toluene mixture than in the solution containing toluene alone. BCF values cannot be calculated because the authors expresssed ^{3}H-toluene uptake as counts per minute per larvae rather than counts per minute per gram. The weight of the larvae was not provided. Interpretation was also complicated by rapid loss of ^{3}H-toluene (half-time about four hours) during the uptake period. It is likely, however, that uptake by ingestion of toluene adsorbed to food particles can be a significant route of accumulation in aquatic organisms.

Ogata and Miyake (1973) identified toluene as the cause of offensive odor in the flesh of grey mullet (Mugil japanicus) taken from a harbor receiving effluents from refineries and petrochemical industries. Toluene was identified in seawater and fish tissue by gas chromatography, infrared (IR) and ultraviolet (UV) absorption, and mass spectrometry. The toluene concentration in most fish was not quantified; however, the flesh of one mullet with an offensive odor contained 5 ppm toluene. Additional experiments showed that toluene was accumulated by caged eels kept for 10 days in several locations in the harbor to an average of 2.4 times the water concentration. These eels had the same offensive odor as mullet collected from the harbor. In another experiment, four eels were exposed in seawater to which a mixed solution of benzene, toluene, and xylenes was added daily for 5 days. The concentration of each chemical was then measured in seawater, muscle, and liver. The results with toluene were as follows:

	Fish No.	Toluene Concentration (ppm)	BCF
Muscle	1	11.2	0.70
	2	2.6	0.16
	3	5.1	0.32
	4	30.8	1.91
	Mean	12.4	0.77
Liver	1	9.0	0.56
	2	2.5	0.16
	3	5.2	0.32
	4	2.5	0.16
	Mean	4.8	0.30
Water	--	16.1	--

The results indicate that BCF in muscle was equal to or greater than the BCF in liver and that tissue concentrations rarely exceeded the water concentration.

In later experiments, Ogata and Miyake (1978) found that eels (Anguilla japonica) accumulated toluene to whole-body concentrations greater than the water concentration in freshwater. For this study, the authors studied the uptake and elimination of toluene by eels exposed in freshwater to crude oil. The animals were exposed for 10 days to a recirculating oil suspension (50 ppm, w/v), which was renewed every day. During this period, the toluene concentration was measured in pooled groups of 5 eels taken on 1, 5, and 10 days after beginning exposure. The concentration of toluene in water was measured each day at 1, 3, 6, 9, 14.5, and 24 hours after preparing the crude oil suspensions. The remaining eels were then transferred to clean seawater and sampled after 3, 5, and 10 days of depuration. The average toluene concentration in water during the uptake period was 0.130 ppm. The concentration in eels was 0.641 ppm after 1 day, 1.547 ppm after 5 days, and 1.718 ppm after 10 days. The respective BCF values were 4.9, 11.9, and 13.2. A semilogarithmic plot of the logarithm of tissue concentration versus time indicated that equilibrium had not quite been reached by 10 days. The depuration phase of the experiment showed that tissue concentration decreased rapidly from 1.718 ppm at the beginning of depuration to 0.315 ppm after 3 days, 0.121 ppm after 5 days, and 0.035 ppm after 10 days. A semilog plot showed that toluene was eliminated in 2 phases. The elimination half-time during the first phase, lasting from 0 to 5 days, was 1.4 days. About 93% of the accumulated toluene was eliminated by the end of this period. The remaining toluene was eliminated at a somewhat slower rate, with about 2% of the accumulated toluene remaining after 10 days of depuration.

The only information found concerning food-chain transfer of toluene is provided by Berry and Fisher (1979), who exposed mosquito larvae (Aedes aegypti) to ^{14}C-toluene for 3 hours and then fed them to bluegill sunfish (Lepomis macrochirus). In duplicate experiments, each of 25 fish in separate containers were fed with 10 contaminated larvae. The mean level of radioactivity in 10 larvae was 736 cpm in the first experiment and 3196 cpm in the second experiment. Internal organs (spleen, gall bladder, liver, stomach, intestine, and kidney) from 5 fish, sampled at each interval of 1, 4, 8, 24, and 48 hours after feeding, were analyzed for radioactivity by liquid scintillation counting. Radioactivity was expressed as counts per minute per organ rather than on a weight basis. The only organ that had counts per minute values significantly greater than background levels was the stomach at 1, 4, and 8 hours after feeding. The authors concluded that an insignificant amount of toluene, if any, leaves the digestive tract to be accumulated in other organs of sunfish. The validity of this conclusion is unknown because the dose was so low that absorption, if it had occurred, could not have been differentiated from background counts and because the counts were not expressed on a tissue weight basis, even in the stomach.

In summary, the available information indicates that the primary path of toluene uptake in aquatic organisms is direct absorption from water. The reported or calculated BCF values for the edible portion or the whole organism ranged from <1 to about 14, indicating that toluene has a low bioconcentration potential. These BCF values are lower than the value predicted on the basis of the relationship established between octanol-water partition coefficient (P) of lipophilic compounds and steady-state BCF (Veith et al., 1979). This relationship, expressed by the equation "log BCF = (0.85 log P) - 0.70," would predict a BCF of 39, using a log P value of 2.69 for toluene (see Subsection 3.4.2.).

Low bioconcentraton potential, rapid depuration, and the ability of fish to metabolize toluene all indicate that toluene is unlikely to biomagnify through aquatic food chains. Aquatic organisms do accumulate toluene, however, and concentrations in edible species from polluted areas have reached levels that cause organoleptic effects in humans (Ogata and Miyake, 1973).

16.3. EFFECTS ON MICROORGANISMS

Toluene has been used for quite some time as an antimicrobial agent. Sabalitschka and Preuss (1954) sterilized a urine sample containing *Escherichia coli* and *Pseudomonas fluorescens* within 24 hours with 4000 mg/ℓ toluene. Threshold concentrations for toluene have been established by Bringmann and Kuhn (1959, 1976, 1980) and Bringmann et al. (1977) for various microorganisms. These investigators reported values of 29 mg/ℓ for *P. putida*, 200 mg/ℓ for *E. coli*, and greater than 450 mg/ℓ for the ciliated protozoan *Uronema parduczi*. Partial sterilization of soil was achieved by adding toluene to the soil (Pochon and Lajudie, 1948).

The effects of toluene on bacterial activity and growth have also been studied. As measured by methane evolution rates, 20 mg/ℓ toluene increased the growth rate of bacteria in sewage sludge deposits, while 200 mg/ℓ produced a toxic effect (Barash, 1957). Similarly low levels of toluene allowed good growth of *P. putida* and *Nocardia* sp., while saturation levels (515 mg/ℓ at 20°C) were toxic (Gibson, 1975). Depending on the concentration (173 to 17,300 mg/ℓ), a rotifer (*Dicranophorus forcipatus*) was unaffected, or temporarily inhibited, or permanently inhibited by toluene (Erben, 1978). Death and disintegration of rumen ciliates occurred between 460 and 645 mg/ℓ of toluene (Eadie et al., 1956). At sublethal concentrations (1000 and 6000 mg/ℓ), toluene caused a negative chemotactic response or totally inhibited the chemotatic response of all marine bacteria tested (Mitchell et al., 1972; Young and Mitchell, 1973). Although the effects were reversible, the authors of the 1972 paper expressed concern that the inhibition could seriously undermine the capacity of the marine microflora to control the self-purification processes in the sea. Beck and Poschenrieder (1963) found that high concentrations of toluene (50 to 100,000 mg/g of soil) suppressed soil microflora activity. In addition, they found that gram-positive bacilli sporeformers, streptomycetes, and cocci were especially resistant, while gram-negative bacteria were sensitive.

Toluene has been shown to affect the integrity of the microbial cell wall and cytoplasmic membrane (Dean, 1978). Thompson and Macleod (1974) reported that marine pseudomonad cells washed and suspended in 0.5 M NaCl were lysed by treatment with 20,000 mg/ℓ toluene and released 95% of the cells' alkaline phosphatase. Because the cells remained intact with 0.05 M $MgSO_4$ and 20,000 mg/ℓ toluene, the authors concluded that Mg ions prevented cellular disruption by strengthening the integrity of the cell wall. Woldringh (1973) established that a 2500 mg/ℓ solution of toluene partially dissolved the inner cytoplasmic membrane of *E. coli* and displaced nuclear material to the periphery of the cell. DeSmet et al. (1978) reported that at 100,000 mg/ℓ toluene, the cytoplasmic membrane was completely disorganized. The presence of Mg ions at lower toluene concentrations (up to 10,000 mg/ℓ), however, prevented extensive damage to the cytoplasmic membrane and loss of intracellular material; thus, permeability depended on the integrity of the outer membrane (DeSmet et al., 1978). Deutscher (1974) found that the effects of toluene treatment were dependent on various cultural conditions including pH, temperature, Mg ion concentration, and age of the culture. Temperature-dependent effects of toluene treatment were also reported by Jackson and DeMoss (1965). Toluene changed the asymmetric unit membrane profile to a symmetric profile in vegetative cells of *Bacillus subtilis* and caused gaps in the membrane to appear (Silva et al., 1978). Fan and Gardner-Eckstrom (1975) found that toluene-treated *Bacillus megaterium* cells liberated a membrane protein essential for peptidoglyca synthesis and that this protein could be added back to the membrane to reconstitute peptidoglycan synthesis. Toluene at 86,000 mg/ℓ induced the autolysis of *Saccharomyces cerevisiae*, the release of UV absorbing substances from the cells, and the deacylation of phospholipids (Ishida-Ichimasa, 1978). At saturation concentrations of toluene, however, no cytolysis of yeast occurred (Lindenberg et al., 1957). Scholz et al. (1959) noted that toluene-treated yeast cells accumulated hexosephosphates. Bucksteeg (1942) found that the concentration of toluene and time of exposure determined its effect on *Cytophaga* sp. and *Azotobacter chroococcum*. The lower the concentration, the longer the contact time needed to produce lethal effects. *Azotobacter* was more resistant than the *Cytophaga* sp. Bucksteeg theorized that toluene affected the physical and chemical constitution of the cell. An alteration in plaque morphology in two coliphages (T_6rt and T_3) occurred with 1% toluene (Brown, 1957).

The ability of toluene to disrupt cell membranes led to the use of this compound as an unmasking agent in microbial research to assay a variety of enzymes (Herzenberg, 1959; Dobrogosz and DeMoss, 1963; Levinthal et al., 1962). The *in vitro* assays using toluene have been used to make enzymes within a cell accessible to exogenous substrates (Jackson and DeMoss, 1965; DeSmet et al., 1978). Generally, toluene treatment makes the cells permeable to low molecular weight compounds (such as deoxynucleoside triphosphate dNTP) and several macromolecules while remaining impermeable to proteins larger than approximately 50,000 daltons (Deutscher, 1974; DeSmet et al., 1978). Several investigators have used these findings to study DNA replication in bacteria (*E. coli*, *B. subtilis*), bacteriophage (*E. coli*, T_4), and diatoms (*Cylindrotheca fusiformis*) after treating the organisms with 0.1 to 1% toluene in solution (Miller et al., 1973; McNicol and Miller, 1975; Moses and Richardson, 1970; Matsushita et al., 1971; Winston and Matsushita, 1975; Sullivan and Valcani, 1976). Other uses of toluene treated cells are in studying the synthesis of heteroribonucleotides, RNA, and peptidoglycan and the repair synthesis of DNA (DeSmet et al., 1978; Moses and Richardson, 1970; Segev et al., 1973; Winston and Matsushita, 1975). Burger (1971) showed that toluene-treated *E. coli* cells continued DNA replication, but only in that chromosomal region that was about to be replicated *in vitro*. Toluene-treated cells can also be used to study the effects of various antibiotics in cell growth and DNA replication (Hein, 1954; Burger and Glaser, 1973).

Although the exact mechanisms of toluene-induced disaggregation of cell membranes are not known, Jackson and DeMoss (1965) state that the mechanisms fall into two classes: (1) a disaggregating (autolytic) enzyme(s), perhaps synthesized in the presence of toluene, or (2) a direct denaturation of cell membrane constituents such as phospholipids; a condition inhibited by stabilizing factors such as divalent cations (e.g., Mg).

16.4. REFERENCES

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17. EFFECTS ON AQUATIC SPECIES

17.1. GUIDELINES FOR EVALUATION

Evaluation of the available information concerning the effects of toluene on aquatic organisms must take into account several factors. A primary consideration for evaluation of toxicity test results is toluene's high volatility. The half-life for volatilization of toluene from a water column 1 m deep has been reported to be between approximately 30 minutes (Mackay and Wolkoff, 1973) and 5 hours (Mackay and Leinonen, 1975). Benville and Korn (1977) analyzed the toluene concentration in test containers during a 96-hour static toxicity test and showed that the percentage of toluene lost was 48% by 24 hours, 53% by 48 hours, and greater than 99% by 72 hours. Korn et al. (1979) reported that toluene was lost at a greater rate from bioassay containers at 12°C (99% loss by 72 hours) than at 8°C (>99% loss by 96 hours) or at 4°C (75% loss by 96 hours). Potera (1975) found that the observed half-life of toluene in bioassay containers was 16.5 ± 1.13 hours. The rate of volatilization of toluene from water varies with the amount of mixing, temperature, surface area to volume ratio, and other factors. Adsorption to sediments and suspended particles may decrease evaporative loss and result in greater persistence of toluene. Although adsorption may lower the concentration of dissolved toluene in the water column, binding to sediment and suspended matter may increase the effective exposure concentration to benthic and filter-feeding organisms.

Most of the reported aquatic toxicity studies with toluene have used a static exposure technique. In most cases, the LC_{50} has been calculated on the basis of initial nominal (unmeasured) or initial measured concentrations. The test organisms in these static experiments, however, are exposed to rapidly decreasing toluene concentrations. Most of the reported acute static toxicity studies show little or no change in the LC_{50} value between 24 and 96 hours. This lack of change indicates that most, if not all, of the mortalities in these tests occurred during the first 24 hours when toluene concentrations were highest. In contrast, those flow-through studies that reported acute LC_{50} values at more than one exposure period showed that LC_{50} values decreased significantly with time.

Numerous other factors may affect the results of toxicity tests with toluene. It has been shown that the acute toxicity of toluene is affected in some cases by temperature and salinity (Section 17.3.). These effects on toxicity may be due to effects on the test organisms (metabolism, uptake, stress, etc.), effects on the physicochemical behavior of toluene (solubility, volatilization, etc.), or interactive effects of both. For example, toluene is less soluble in saltwater than in freshwater and is both more soluble and more volatile at higher temperatures. Laboratory results may also be influenced by the loading ratio (gram organism per liter water); dissolved oxygen concentration; age, health, and species of test organisms; and other exposure conditions, all of which may interact to affect the results in an unpredictable manner.

Prediction of environmental effects from laboratory results must consider the influence of the variables associated with laboratory tests and with the natural variability intrinsic to the aquatic environment. Results of static acute toxicity tests with volatile compounds such as toluene may approximate the acute toxic effects that may occur in nature to the same species during accidental spills, because toluene concentrations rapidly decrease in both situations. Flow-through acute toxicity tests may provide some insight into the expected effects of a short-term but constant release of toluene into the aquatic environment, as might occur in areas receiving refinery or petrochemical effluents. Neither static nor flow-through acute toxicity tests can predict the chronic effects of low level toluene pollution. In addition, acute toxicity tests usually determine the concentration of toxicant that kills or affects 50% of the test population. LC_{50} or EC_{50} values, therefore, represent concentrations that are toxic to half the population, and provide no information concerning the concentration that will have no adverse effects during acute or chronic exposure.

17.2. EFFECTS OF ACCIDENTAL SPILLS

No information was found concerning the effects of accidental spills of toluene per se on aquatic organisms; however, toluene is one of the major aromatic components of crude oil and such refined petroleum products as diesel fuel, gasoline, and jet fuel, all of which have been released in large amounts to the aquatic environment during spills.

The long term ecological impact of accidental spills of toluene is unknown. In spill situations, most of the toluene would probably evaporate rapidly. For instance, McAuliffe (1976) reported that toluene, benzene, and xylene could be found in the water under crude oil slicks only during the first 30 minutes after spillage. In contrast, spills in areas of shallow water and restricted water flow, such as in certain portions of estuaries, lakes, and streams, have a greater potential for causing acute mortalities because the toluene may reach higher dissolved concentrations and may persist longer through adsorption to sediments. Toluene is acutely toxic to many aquatic species at concentrations well below its water solubility, and lethal exposure may occur during spills in shallow water.

Although chronic, low-level pollution by toluene has been reported in a Japanese river (Funasaka et al., 1975) and a harbor (Ogata and Miyake, 1973) that received refinery and petrochemical effluents, the effects of such low level chronic pollution in natural aquatic habitats are unknown.

17.3. LABORATORY STUDIES OF TOXICITY

17.3.1. Lethal Effects. The lethal effects of toluene have been reported for numerous species of freshwater and marine fish and invertebrates. The acute LC_{50} for 22 species of freshwater and marine animals ranged between 3 and 1180 ppm (Table 17-1). All but six of the LC_{50} values were determined in static tests. Of the six flow-through LC_{50} tests, four utilized measured toluene concentrations. No information was found concerning the effects of toluene on amphibians.

17.3.1.1. FRESHWATER FISH -- The earliest investigation of toluene toxicity to freshwater fish was conducted by Shelford et al. (1917), who reported that 1 hour of exposure to 61 to 65 mg/ℓ toluene was lethal to orange spotted sunfish (Lepomis humilis). This test was conducted under static conditions at 20°C in freshwater of unspecified temperature and composition.

Degani (1943) conducted static toxicity tests with 15-day-old lake trout (Salvelinus namaycush) fry and 1.5 g mosquitofish (Gambusia affinis) in dechlorinated tapwater at 17 to 18°C using 3 to 5 fish per container (2 ℓ volume). The time to death at a nominal exposure concentration of 90 ppm toluene was 390 minutes for trout and 47 minutes for mosquitofish. The time to death of trout fry exposed to 50 ppm toluene was 258 minutes.

Wallen et al. (1957) also conducted static acute toluene toxicity tests with female mosquitofish (Gambusia affinis) of unspecified size in turbid pond water (150 ppm turbidity as measured by Jackson turbidimeter, pH 7.5 to 8.5, methyl orange alkalinity < 100 ppm, temperature 17 to 22°C). For these toxicity tests, ten fish per concentration were added immediately after addition of different amounts of toluene to the bioassay containers (15 liter volume). The test solutions were constantly aerated and mortalities were recorded daily for 96 hours. The 24, 48, and 96 hour LC_{50} values were 1340, 1260, and 1180 ppm, respectively. These values were estimated on the basis of the initial nominal

TABLE 17-1

Acute Toxicity of Toluene to Fish and Aquatic Invertebrates

Species	Temp. (°C)	Type Test	LC50 24 h	LC50 48 h	LC50 72 h	LC50 96 h	No Effect Concentration	Reported Concentration Units	Comments	Reference
FISH										
Freshwater										
Ide	20±1	SU	---	70	---	---	52	mg/ℓ	Lab 1, 100% kill at 88 mg/ℓ.	Juhnke and Ludemann, 1978
(*Leuciscus idus melanotus*)	20±1	SU	---	422	---	---	365		Lab 2, 100% kill at 470 mg/ℓ. Tests were supposedly conducted under identical conditions.	
Mosquitofish (*Gambusia affinis*)	17 to 22	SU	1340	1260	---	1180	560	ppm	Tests were conducted in aerated turbid pond water.	Wallen et al., 1957
Goldfish (*Carassius auratus*)	20±1	SM	58	---	---	---	---	mg/ℓ	Test was conducted in tap water (pH 7.8)	Bridie et al., 1979
Goldfish (*Carassius auratus*)	25	SU	57.7 (48.9 to 68.8)	57.7 (48.9 to 68.8)	---	57.7 (48.9 to 68.8)	---	mg/ℓ	Test was conducted in soft water.	Pickering and Henderson, 1966
Goldfish (*Carassius auratus*)	17 to 19	FM	41.6 (32.0 to 71.7)	27.6 (21.6 to 36.0)	25.3 (20.1 to 31.9)	22.80 (17.1 to 30.0)	--- to	ppm	Tests were conducted under flow-through conditions in soft dechlorinated tap water. The test was continued to 720 h (30 d) at which time the LC_{50} (and 95% confidence interval) was 14.6 (10.7 to 20.0) ppm.	Brenniman et al., 1976
Fathead minnow (*Pimephales promelas*)	25	FM	---	---	---	18-72	---	mg/ℓ	Embryos were more resistant than larvae	Devlin et al., 1982
Fathead minnow (*Pimephales promelas*)	25	SU	46.3 (37.0 to 59.4)	46.3 (37.0 to 59.4)	---	34.3 (22.8 to 45.9)	---	mg/ℓ	Tests were conducted in soft water.	Pickering and Henderson, 1966
Fathead minnow (*Pimephales promelas*)	25	SU	56.0 (44.7 to 67.1)	56.0 (46.7 to 67.1)	---	42.3 (33.5 to 53.5)	---	mg/ℓ	Tests were conducted in hard water.	

TABLE 17-1 (cont.)

Species	Temp. (°C)	Type Test	LC_{50}				No Effect Concentration	Reported Concentration Units	Comments	Reference
			24 h	48 h	72 h	96 h				
Bluegill sunfish (Lepomis macrochirus)	25	SU	24.0 (18.9 to 30.5)	24.0 (18.9 to 30.5)	---	24.0 (18.9 to 30.5)	---	mg/ℓ	Tests were conducted in hard water.	Pickering and Henderson, 1966
Bluegill sunfish (Lepomis macrochirus)	NR	SU	16.6 (15.0 to 19.1)	13.3 (11.6 to 14.8)	12.7 (11.5 to 14.5)	12.7 (11.5 to 14.5)	10.0	ppm	Only these data cited in U.S. EPA, 1980.	U.S. EPA, 1978
Guppies (Poecilia reticulata)	25	SU	62.8 (55.0 to 73.7)	61.0 (52.8 to 71.9)	---	59.3 (50.9 to 70.3)	---	mg/ℓ	Tests were conducted in hard water.	Pickering and Henderson, 1966
Zebrafish (Brachydanio rerio)	20±1	FU	---	25 to 27	---	---	---	mg/ℓ	Tests were conducted in closed aquaria with dechlorinated hard tap water at a flow rate of 6 ℓ/h.	Sloof, 1978 Sloof, 1979
Medaka (Oryzias latipes)	25±2	SU	80 (mean= 80)	20 to 135 (mean= 63)		23 to 110 (mean= 54)	≤16	mg/ℓ	Range and mean of LC_{50} values for different stage embryos	Stoss and Haines, 1979
Medaka (Oryzias latipes)	25±2	SU	44	36		32	---	mg/ℓ	LC_{50} values for fry. The 168 h. LC_{50} was 23 mg/ℓ.	Stoss and Haines, 1979
Coho salmon fry (Oncorhynchus kisutch)	--- ---	FM FM	--- ---	--- ---	--- ---	9.36 3.08	--- ---	μℓ/ℓ μℓ/ℓ	Unparasitized Parasitized	Moles, 1980 Moles, 1980
MARINE										
Coho salmon (Oncorhynchus kisutch)	8	SU	---	22.4	22.4	22.4	10	ppm	Tests were conducted in artificial saltwater (pH 8.1, 30°/oo salinity).	Morrow et al., 1975
Pink salmon fry (Oncorhynchus kisutch)	12	SM	5.4 (4.4 to 6.5)	---	---	---	---	ppm	Tests were conducted according to methods of Korn et al., 1979.	Thomas and Rice, 1979

TABLE 17-1 (cont.)

Species	Temp. (°C)	Type Test	LC$_{50}$ 24 h	LC$_{50}$ 48 h	LC$_{50}$ 72 h	LC$_{50}$ 96 h	No Effect Concentration	Reported Concentration Units	Comments	Reference
Pink salmon (Oncorhynchus kisutch)	4	SM	---	---	---	6.41 (5.73 to 7.18)	---	μℓ/ℓ	Tests were conducted with salmon fry acclimated to 28^{o}/oo seawater at different temperatures.	Korn et al., 1979
	8	SM	---	---	---	7.63 (6.86 to 8.48)	---			
	12	SM	---	---	---	8.09 (7.45 to 8.78)	---			
Striped bass (Morone saxatilis)	16	SM	7.3	---	---	7.3	---	μℓ/ℓ	Tests were conducted in 25^{o}/oo salinity seawater with juvenile fish.	Benville and Korn, 1977
Sheepshead minnow (Cyprinodon variegatus)	NR	SU	>277 <485	>277 <485	---	>277 <485	277	ppm	Data only cited in U.S. EPA, 1980.	U.S. EPA, 1978
Sheepshead minnow (Cyprinodon variegatus)	29	FM	---	---	---	13 (5.0 to 35)	---	mg/ℓ	Tests were conducted in 15% salinaty seawater wih juvenile fish.	Ward et al., 1981
INVERTEBRATES										
Freshwater										
Water flea (Daphnia magna)	22±1	SU	310 (240 to 420)	310 (240 to 420)	---	---	28	mg/ℓ	Test was conducted with reconstituted wel' water (hardness 72±6 mg/ℓ as $CaCO_3$, pH 7.0±0.2) in containers sealed with plastic wrap.	LeBlanc, 1980
Water flea (Daphnia magna)	23	SU	---	60	---	---	---	mg/ℓ	Test was conducted in natural water (pH 7.5, hardness 214 mg/ℓ).	Bringmann and Kuhn, 1959
Mosquito larvae (Aedes aegypti)	25±1	SM	21.52 (21.36 to 21.68)	---	---	---	9.95	ppm	Test was conducted with distilled water.	Berry and Brammer, 1977
Marine										
Brine shrimp nauplii (Artemia salina)	24.5	SU	33	---	---	---	---	mg/ℓ	Test was conducted with artificial seawater.	Price et al., 1974

TABLE 17-1 (cont.)

Species	Temp. (°C)	Type Test	LC_{50} 24 h	LC_{50} 48 h	LC_{50} 72 h	LC_{50} 96 h	No Effect Concentration	Reported Concentration Units	Comments	Reference
Bay shrimp (*Crago franciscorum*)	16	SM	12 (10 to 13)	---	---	4.3 (3.1 to 5.8)	---	µℓ/ℓ	Tests were conducted with 25°/oo salinity seawater.	Benville and Korn, 1977
Shrimp (*Eualus* spp.)	4	SM	---	---	---	21.4 (19.5 to 23.5)	---	µℓ/ℓ		Korn et al., 1979
	8	SM	---	---	---	20.2 (17.9 to 22.8)		µℓ/ℓ		Korn et al., 1979
	12	SM	---	---	---	14.7 (13.1 to 16.6)		µℓ/ℓ		Korn et al., 1979
Grass shrimp (*Pacaemonetes pugio*)	20	SM	20.2 (16.3 to 22.5)	---	---	---	---	mg/ℓ	Adults at 15°/oo salinity.	Potera, 1975
	20	SM	17.2 (14.9 to 19.4)	---	---	---	---	mg/ℓ	Adults at 25°/oo salinity.	Potera, 1975
	10	SM	37.6 (35.0 to 40.3)	---	---	---	---	mg/ℓ	Adults at 15°/oo salinity.	Potera, 1975
	10	SM	38.1 (36.1 to 39.6)	---	---	---	---	mg/ℓ	Adults at 25°/oo salinity.	Potera, 1975
Grass shrimp (*Pacaemonetes pugio*)	20	SM	30.6 (21.3 to 44.5)	---	---	---	---	mg/ℓ	Larvae at 15°/oo salinity.	Potera, 1975
	20	SM	25.8 (18.8 to 34.6)	---	---	---	---	mg/ℓ	Larvae at 25°/oo salinity.	Potera, 1975

TABLE 17-1 (cont.)

Species	Temp. (°C)	Type Test	LC$_{50}$ 24 h	LC$_{50}$ 48 h	LC$_{50}$ 72 h	LC$_{50}$ 96 h	No Effect Concentration	Reported Concentration Units	Comments	Reference
Grass shrimp (Palaemonetes pugio)	NR	SU	---	---	---	9.5	---	mg/ℓ	---	Neff et al., 1976
Mysid shrimp (Mysidopsis bahia)	NR	SU	64.8 (50.9 to 82.5)	56.3 (43.0 to 70.8)	56.3 (43.0 to 70.8)	56.3 (43.0 to 70.8)	27.7	ppm	Data only cited in U.S. EPA, 1980.	U.S. EPA, 1978
Dungeness crab (Cancer magister)	NR	FU	---	170	---	28	---	mg/ℓ	Larvae.	Caldwell et al., 1976
Copepod (Nitocra spinipes)	20	SM	24.2 (19.8 to 30.2)	---	---	---	---	mg/ℓ	15^{o}/oo salinity.	Potera, 1975
	20	SM	74.2 (52.0 to 100.5)	---	---	---	---	mg/ℓ	25^{o}/oo salinity.	Potera, 1975
Pacific oyster (Crassostrea gigas)	20 to 21.5	SU	---	1050	---	---	---	mg/ℓ	Larvae.	Legore, 1974

Temp. = temperature; h = hour; d = day; NR = not reported.

toluene concentrations. Because the test containers were vigorously aerated, it is probable that the actual toluene concentrations decreased rapidly during the exposure period. It was also observed that the turbidity of the toluene solutions decreased from 150 to 100 ppm over the 96-hour exposure period. At concentrations of 560 ppm and below, all fish appeared to be unaffected. The remainder of the test results are presented below:

Concentration (ppm)	Percent Mortality (N = 10)		
	24 h	48 h	96 h
≤ 560	0	0	0
1,000	20	30	40
1,800	80	80	100
3,200	80	90	100
5,600	100	100	100
10,000	100	100	100

Pickering and Henderson (1966) investigated the acute toxicity of toluene to fathead minnows (Pimephales promelas), bluegill sunfish (Lepomis macrochirus), goldfish (Carassius auratus), and guppies (Lebistes reticulatus = Poecilia reticulata). The length and weight of the fish used for testing were 3.8 to 6.4 cm and 1 to 2 g for the first 3 species and 1.9 to 2.5 cm and 0.1 to 0.2 g for guppies. Each test utilized 10 fish per concentration or control in either 10 ℓ (minnows, sunfish, goldfish) or 2 ℓ (guppies) of soft water (pH 7.5, alkalinity 18 mg/ℓ, EDTA hardness 20 mg/ℓ) made by mixing 5 parts of hard natural spring water with 95 parts of distilled demineralized water. In addition, fathead minnows were tested (10 fish/concentration) in the hard spring water (pH 8.2, alkalinity 300 mg/ℓ, EDTA hardness 360 mg/ℓ) to investigate the effect of these water characteristics on toluene toxicity. All tests were conducted at 25°C. The test solutions were not aerated, and dissolved oxygen concentrations were measured but not reported. The 24, 48, and 96-hour LC$_{50}$ values and their 95% confidence limits, as calculated by the moving average-angle method of Harris (1959) using initial nominal toluene concentrations, are presented in Table 17-1. The 96-hour LC$_{50}$ values increased in the order of bluegill sunfish (24.0 mg/ℓ), fathead minnow (34.3 mg/ℓ in soft water, 42.3 mg/ℓ in hard water), goldfish (57.7 mg/ℓ), and guppies (59.3 mg/ℓ). The 96-hour LC$_{50}$ for fathead minnows in soft water was not significantly different from the 96-hour LC$_{50}$ for the same species in hard water. Comparison of the 95% confidence limits of the 96-hour LC$_{50}$ values in soft water for the 4 species indicated that the LC$_{50}$ values were not significantly different between fathead minnows and bluegill sunfish or between goldfish and guppies. Both fathead minnows and bluegill sunfish had 96-hour LC_{50} values significantly lower than goldfish and guppies. The 96-hour LC_{50} was not significantly different from the 24-hour LC_{50} for any of the species tested in soft water.

Replicate flow-through acute toxicity tests were also conducted with fathead minnow embryos, 1-day-old protolarvae, and 30-day-old larvae by Devlin et al. (1982). The 96-hour LC_{50} ranged between 18 and 31 mg/ℓ for 30-day-old fish, between 25 and 36 mg/ℓ for protolarvae,and between 55 and 72 mg/ℓ for embryos. Embryos were significantly more resistant that the other life stages.

Static acute LC_{50} values for bluegill sunfish have also been reported by the U.S. EPA (1980). The 24, 48, 72, and 96-hour LC_{50} values were 16.6, 13.3, 12.7, and 12.7 ppm, respectively. No effects were observed at or below 10 ppm. Additional information concerning these tests was not available.

Berry (1980) mentioned that the upper non-lethal toluene concentration for bluegill sunfish (*Lepomis macrochirus*) was 8.7 mg/ℓ. The duration of exposure and lowest lethal concentration were not specified.

Bridie et al. (1979) and Brenniman et al. (1976) also investigated the acute toxicity of toluene to goldfish. Bridie et al. (1979) used goldfish of slightly greater weight (mean 3.3 g, range 2.3 to 4.3 g) than Pickering and Henderson (1966) to determine the static 24-hour LC_{50}. In this test, 6 fish per concentration were exposed without aeration to a toluene series in 25 ℓ of tapwater that had a pH of 7.8 and contained (in milligrams per liter): $Cl^- = 65$; $NO_2^- = 0$; $NO_3^- = 4$; $SO_4^{2-} = 35$; $PO_4^{3-} = 0.15$; $HCO_3^- = 25$; $SiO_2 = 25$; $NH_4^+ = 0$; Fe = 0.05; Mn = 0; $Ca^{2+} = 100$; $Mg^{2+} = 8$; and alkali as $Na^+ = 30$. The toluene concentration was measured at the beginning and end of the test. The 24-hour LC_{50}, obtained by interpolation from a graph of the logarithm of concentration versus percent mortality, was 58 mg/ℓ, which is the same as the 24-hour LC_{50} for goldfish reported by Pickering and Henderson (1966).

Much larger goldfish (length, 13 to 20 cm; weight, 20 to 80 g) were used by Brenniman et al. (1976) to determine the acute toxicity of toluene under flow-through exposure conditions. The LC_{50} values were determined by exposing 6 fish per 38 ℓ aquarium to three toluene concentrations (and a control) in dechlorinated soft tapwater (methyl orange alkalinity = 34 ppm as $CaCO_3$; phenolphthaline alkalinity = 37 ppm as $CaCO_3$; total hardness = 80 ppm as $CaCO_3$; calcium = 21.6 ppm; magnesium = 5.3 ppm; SiO_3 = 8 ppm; chromium = <0.002 ppm; pH 7.0 ± 0.3;

temperature 17 to 19°C) at a flow rate calibrated to renew the test chamber volumes every 1.5 hours. This flow rate was sufficient to maintain dissolved oxygen concentrations at ≥ 7 ppm and to maintain constant toluene concentrations, as measured by continuous monitoring at 210 nm by spectrophotometer. The 24, 48, 72, and 96-hour LC_{50} values, calculated by probit analysis, were 41.6, 27.6, 25.3, and 22.8 ppm, respectively. Although most of the fish died during the first 24 hours, the 96-hour LC_{50} was significantly lower than the 24-hour LC_{50}. These LC_{50} values are somewhat lower than those reported by Pickering and Henderson (1966) and Bridie et al. (1979) for goldfish tested under static conditions. In addition, the LC_{50} values reported by Pickering and Henderson (1966) did not decrease significantly from 24 to 96 hours. These differences are probably due to a rapid decline in the toluene concentration through evaporation in the static tests in contrast to constant toluene concentrations in the flow-through test. Brenniman et al. (1976) continued their flow-through exposure test for 30 days, at which time the LC_{50} had decreased to 14.6 ppm. These results emphasize the fact that static acute toxicity tests may seriously underestimate the acute toxicity of toluene and that chronic effects may occur at concentrations that are considerably lower than those that cause acute effects.

Juhnke and Ludemann (1978) investigated the static acute toxicity of toluene to the ide (*Leuciscus idus melanotus*) using comparable procedures in two different laboratories. The toxicity tests were conducted according to the methods of Mann (1975, 1976), i.e. 48 hours of exposure with 10 fish (1.5 ± 0.3 g, 5 to 7 cm) per concentration in tapwater (pH 7-8, hardness 268 ± 54 mg/ℓ) at 20 ± 1°C. The 48 hour LC_0 (0% mortality), LC_{50}, and LC_{100} (100% mortality) values determined at each laboratory were as follows:

	48 Hour Lethal Concentration Values (mg/ℓ)		
	LC_0	LC_{50}	LC_{100}
Laboratory 1	52	70	88
Laboratory 2	365	422	470

Although it was stated that these tests were conducted under comparable conditions, the results were clearly different. The concentration that caused no deaths of fish in laboratory 2 (365 mg/ℓ) was about 4 times higher than the concentration that killed all fish in laboratory 1 (88 mg/ℓ). The authors did not discuss the reasons for the difference in results.

Sloof (1978, 1979) reported that the 48-hour LC_{50} of toluene to zebrafish (Brachydanio rerio) was 25 to 27 mg/ℓ. This test was conducted under flow-through (6 ℓ/hr) exposure conditions using 10 fish per concentration in 10 ℓ sealed aquaria and dechlorinated tapwater (20 ± 1°C; pH 8.0 ± 0.2; hardness 180 ± 1.8 mg/ℓ as $CaCO_3$).

The acute effects of toluene on parasitized and unparasitized coho salmon (Oncorhynchus kisutch) fry were studied by Moles (1980). The parasitized fry were artificially infected before toluene exposure with glochidial larvae of the freshwater mussel, Anodonta oregonensis. Toluene exposure was conducted under flow-through conditions, using five measured concentrations and 20 fish per concentration. The temperature and characteristics of the water used were not specified. The 96 hour LC_{50}, as calculated by probit analysis, was 9.36 μℓ/ℓ (ppm) for unparasitized fish and 3.08 μℓ/ℓ for fish parasitized with a mean number of 69 glochidia per fish. The LC_{50} values were significantly different, indicating that parasitized fish were less resistant to the effects of toluene.

Stoss and Haines (1978) investigated the effects of static exposure to toluene on the survival of fertilized eggs and newly hatched fry of the medaka, Oryzias latipes. Groups of ten eggs or fry were exposed in loosely capped vials containing 20 mℓ of the exposure medium (synthetic rearing medium: pH 7.6; akalinity 99 mg/ℓ as $CaCO_3$) at 23 ± 2°C. Toluene concentrations were prepared by diluting a water-soluble extract of 10 mℓ toluene/ℓ medium. In order to determine the sensitivity of different stages of embryo development, tests were begun with eggs of various ages after fertilization. Tests with fry were all begun within 24 hours after hatching. Nominal initial toluene concentrations were used for calculation of LC_{50} values. The LC_{50} values for embryos varied with length of exposure and the age at time of introduction. The mean 24, 48, and 96-hour LC_{50} values for all ages of embryos were 80, 63, and 54 mg/ℓ. The range of LC_{50} values was 20 to 135 mg/ℓ at 48 hours and 23 to 110 mg/ℓ at 96 hours (Stoss, personal communication). Early ($\leq$3.5 hours old) and late ($\geq$192 hours old) embryos had significantly lower LC_{50} values at each exposure period than embryos of intermediate age at time of introduction. The 24, 48, 96, and 168-hour LC_{50} values for fry were 44, 36, 32, and 23 mg/ℓ, respectively (Stoss, personal communication). These values were lower than the mean embryo LC_{50} values for the same exposure period; however, fry LC_{50} values were greater than the LC_{50} values for the susceptible early and late stage embryos and lower than most of the LC_{50} values for intermediate stage embryos. Stoss and Haines (1978) also investigated the sublethal effects of toluene on hatching time and induction of developmental abnormalities. These sublethal effects are discussed in Section 17.3.2.1.

17.3.1.2. MARINE FISH -- Morrow et al. (1975) studied the effects of toluene on young coho salmon (Oncorhynchus kisutch) that had been acclimated to artificial seawater (30 °/oo (parts per thousand) salinity; 8°C; pH 8.1) for up to 2 weeks. A static exposure technique was used in which toluene was added directly to exposure aquaria containing fish and 73 ℓ of seawater ($\leq$1 g fish/ℓ water) to give nominal concentrations of 0, 1, 10, 50, and 100 ppm toluene. The average weight of the fish used during triplicate tests ranged from 5 g/fish in the fall of the year to nearly 40 g/fish in the spring. The mortality data provided in the paper are given below:

Concentration (ppm)	No. of Tests	No. of Fish per Concentration	Percent Mortality				
			0 h	24 h	48 h	72 h	96 h
0	3	30	0	7	7	13	13
1	3	30	0	7	7	13	13
10	3	30	0	0	0	3	10
50	1	10	0	90	100	100	100
100	3	30	0	93	100	100	100

Using 2 x 2 contingency table analysis, the authors determined that mortality was significantly different from control mortality at 50 and 100 ppm, but not at 10 and 1 ppm. The reasons for control mortality were not discussed but may have been due to salinity stress; the authors mentioned that smaller fish adapted less easily to seawater than larger fish. In order to incorporate these data into Table 17-1, the LC_{50} values were calculated as the geometric mean of 50 ppm (mortality = 100%) and 10 ppm (mortality corrected for control mortality = 0%). This value for the 48, 72, and 96-hour LC_{50} was 22.4 ppm. The authors state that fish exposed to 50 and 100 ppm toluene exhibited rapid, violent, and erratic swimming within 15 to 20 minutes, followed by "coughing," loss of equilibrium, and death of most fish within the first few hours.

The acute effects of toluene on another species of salmon in seawater were investigated by Korn et al. (1979). Pink salmon (Onchorhynchus gorbuscha) fry, weighing about 0.35 g each, were acclimated to natural seawater (6 to 8°C; 26 to 28 °/oo salinity). Groups of fry were then acclimated to 4, 8, or 12°C for determination of the 96-hour LC_{50} at 3 temperatures. Each toxicity test was conducted with 10 to 15 fry per concentration (<1 g fish/ℓ water). Fish were added to the test containers after addition of an appropriate amount of toluene in water stock solution. The containers were not aerated until after the first 48 hours of exposure to minimize evaporative loss. Even so, analysis showed that toluene decreased to nondetectable levels by 72 hours at 12°C and by 96 hours at 8°C and to 25% of the initial concentration by 96 hours at 4°C. The 96-hour LC_{50} values, estimated by probit analysis using initial measured concentrations expressed as µℓ/ℓ toluene (= ppm), were 6.4 at 4°C, 7.6 at 8°C, and 8.1 at 12°C. The 95% confidence intervals of the 4°C and 12°C LC_{50} values did not overlap, indicating that temperature affected the toxicity of toluene. There was no significant difference between 24 and 96-hour LC_{50} values because almost all deaths occurred within the first 24 hours of exposure. The effect of temperature may have been caused by greater sensitivity of the fish at the lower temperature and/or by the longer persistence of toluene at the lower temperature.

Thomas and Rice (1979) used the previously described techniques of Korn et al. (1979) to determine the static 24-hour LC_{50} of toluene with somewhat larger (1 to 2 g, 4.5 to 5.5 cm) pink salmon fry at 12°C in seawater. The 24-hour LC_{50} (and 95% confidence interval) was 5.4 (4.4 to 6.5) ppm, which is significantly different from the 96-hour LC_{50} value of 8.1 ppm (7.5 to 8.8) obtained with younger fry at 12°C by Korn et al. (1979). The reasons for this difference cannot be determined from the information provided.

A similar static exposure technique was used by Benville and Korn (1977) in their study of the acute toxicity of toluene to juvenile striped bass (Morone saxatilis) in seawater (25 °/oo salinity, 16°C). The test was initiated by adding different amounts of saturated toluene in water stock solution to the test aquaria, each containing 10 fish. Toluene concentrations were measured at the beginning of the test and every 24 hours thereafter to the end of the test. The 24 and 96-hour LC_{50} values were both 7.3 µℓ/ℓ (ppm). Almost all mortalities occurred within 6 hours. The average percent loss of toluene was 40% by 24 hours, 53% by 48 hours, and >99% by 72 hours.

The only flow-through toxicity test with marine fish was conducted by Ward et al. (1981). The flow-through 96-hour LC_{50}, based on measured concentrations, was 13 mg/ℓ for juvenile sheepshead minnows (Cyprinodon variegatus). This value was much lower than that obtained with static tests. The static 96-hour LC_{50} for similar fish was reported to be >277 <485 ppm in an unpublished U.S. EPA study (1978, cited in U.S. EPA, 1980) and in Ward et al. (1981). Although toluene concentrations were not measured in the static test, the difference in LC_{50} values is almost certainly due to rapid loss of toluene from the static test containers.

17.3.1.3. FRESHWATER INVERTEBRATES -- Berry and Brammer (1977) investigated the acute static toxicity of toluene to fourth-instar larvae of the mosquito, *Aedes aegypti*. The larvae were reared from eggs and tested in distilled water at 25 ± 1°C. For each of four replicate tests, duplicate groups of 20 larvae each were exposed to 14 toluene concentrations. The mortality data were pooled (160 larvae/concentration) to calculate the 24-hour LC_{50} by probit analysis. Initial exposure concentrations were determined by gas-liquid chromatography. The 24-hour LC_{50} (± standard error) was 21.52 ± 0.16 ppm. The highest concentration (± standard error) that caused no mortality over the 24-hour exposure period was 9.95 ± 1.30 ppm.

Berry (1980) mentioned that the upper non-lethal toluene concentration for crayfish (*Orconetes rusticus*) was 104.4 mg/ℓ. The duration of exposure and lowest lethal concentration were not specified.

The acute toxicity of toluene has also been determined with the cladoceran, *Daphnia magna*, by Bringmann and Kuhn (1959) and by LeBlanc (1980). Bringmann and Kuhn (1959) reported a 48-hour LC_{50} of 60 mg/ℓ. This static test was conducted with first instar (<24 hours old) *Daphnia magna* in natural freshwater (pH 7.5; hardness 214 mg/ℓ) at 23°C.

LeBlanc (1980) conducted static tests with first instar (<24 hours old) animals in deionized well water reconstituted to a total hardness of 72 ± 6 mg/ℓ as $CaCO_3$ and a pH of 7.0 ± 0.2 at 22 ± 1°C. Three groups of 5 daphnids each were exposed to each of at least five toluene concentrations and uncontaminated water in covered 250 mℓ beakers containing 150 mℓ of test solution. The 24 and 48-hour LC_{50} values (and 95% confidence intervals), based on initial nominal concentrations, were both 310 (240 to 420) mg/ℓ. The "no discernible effect concentration" was 28 mg/ℓ. This LC_{50} value is considerably higher than that reported by Bringmann and Kuhn (1959). The reasons for this difference cannot be determined from the data provided.

17.3.1.4. MARINE INVERTEBRATES -- Price et al. (1974) determined the static 24-hour LC_{50} of toluene to brine shrimp nauplii (*Artemia salina*) in artificial seawater (27.87 g/ℓ NaCl; 1.36 g/ℓ $CaSO_4$; 3.17 g/ℓ $MgSO_4 \cdot 7H_2O$; 8.42 g/ℓ $MgCl_2$; 0.79 g/ℓ KCl; 0.16 g/ℓ $MgBr_2 \cdot 6H_2O$) at 24.5°C. Groups of 30 to 50 newly hatched brine shrimp were exposed to 5 toluene concentrations in 100 mℓ seawater. The estimated 24-hour LC_{50}, based on initial nominal concentrations, was 33 mg/ℓ.

Bay shrimp (Crago franciscorum) were shown by Benville and Korn (1977) to be somewhat more sensitive to toluene. The 24-hour static LC_{50}, determined in natural seawater (25 °/oo salinity) at 16°C, was 12 μℓ/ℓ (ppm). The 96-hour LC_{50} for this species (4.3 μℓ/ℓ) was significantly lower than the 24-hour LC_{50} (non-overlapping 95% confidence limits). These values were calculated from initial measured toluene concentrations.

Korn et al. (1979) investigated the effects of temperature on the acute toxicity of toluene to another genus of shrimp (Eualus spp.). Shrimp (0.8 g; 6 cm long) were acclimated to the test temperatures in natural 26 to 28 °/oo salinity seawater for 4 days and then exposed in groups of 10 to 15 animals to a series of toluene concentrations, prepared by dilution of a saturated water solution. The tissue loading in the test containers was less than 1 g/ℓ. Measurement by UV spectrophotometry showed that toluene concentrations decreased to nondetectable levels by 72 hours at 12°C and by 96 hours at 8°C, and to 25% of the initial concentration by 96 hours at 4°C. The 96-hour LC_{50} values, calculated from initial measured toluene concentrations, were 21.4 μℓ/ℓ at 4°C, 20.2 μℓ/ℓ at 8°C, and 14.7 μℓ/ℓ at 12°C. The 96-hour LC_{50} values at 4°C and 8°C were not significantly different (overlapping 95% fiducial limits) from each other, but both were significantly higher than the 96-hour LC_{50} at 12°C. This trend of greater toxicity at higher temperatures was opposite to the relationship found by these authors for pink salmon fry (Section 17.3.1.2.) and by Potera (1975) for grass shrimp (see below). The reasons for this difference could not be established but may have been due to some combination of effects of temperature on persistence of toluene in water, altered toluene uptake and metabolic rates, and possible interaction of toluene toxicity and temperature stress. The authors concluded that temperature affected the toxicity of toluene to these species of shrimp and salmon but that it would be impossible to predict the effects of temperature change on the toxicity of toluene to other species.

Potera (1975) investigated the effects of temperature (10 and 20°C), salinity (15 and 25 °/oo), and life stage (larvae and adults) on the static 24-hour LC_{50} of toluene to the grass shrimp, Palaemonetes pugio. The 24-hour LC_{50} values, based on measured initial concentrations, ranged from 17.2 to 38.1 mg/ℓ.

As shown by overlapping 95% confidence intervals (Table 12-1), there was no significant difference in LC_{50} values between adults and larvae at the same salinity and temperature, or between adults tested at the same temperature but at different salinities. The LC_{50} was significantly lower at 20°C, however, than at 10°C for adults tested at either 15 ^{o}/oo or 25 ^{o}/oo salinity. The time to produce narcosis in at least 50% of adult shrimp at 20°C was less than 30 minutes at initial exposure concentrations of 19.8 mg/ℓ and greater. Recovery of more than 90% of exposed shrimp could occur if shrimp were transferred to clean water after exposure to up to 30 mg/ℓ for 30 minutes.

Potera (1975) also determined the 24-hour LC_{50} for the copepod, Nitocra spinipes, at a temperature of 20°C and at salinities of either 15 ^{o}/oo or 25 ^{o}/oo. The 24-hour LC_{50} values from replicate tests were 24.4 at 15 ^{o}/oo salinity and 74.2 mg/ℓ at 25 ^{o}/oo salinity. These values were significantly different (non-overlapping 95% confidence intervals). Potera (1975) suggested that the lower salinity may have stressed the copepods, resulting in a lower LC_{50} value.

Neff et al. (1976) also determined the static 96-hour LC_{50} of toluene to grass shrimp, Palaemonetes pugio. This value, based on initial nominal concentrations, was 9.5 mg/ℓ, which is lower than the 24-hour LC_{50} values reported by Potera (1975).

Caldwell et al. (1976) determined the 48 and 96-hour LC_{50} of toluene to larval stages of the dungeness crab (Cancer magister) under flow-through exposure conditions. The 48 and 96-hour LC_{50} values were 170 and 28 mg/ℓ, respectively.

Static acute LC_{50} values for mysid shrimp (Mysidopsis bahia) have been reported by the U.S. EPA, (1980). The 24 and 48 to 96-hour LC_{50} values were 64.8 and 56.3 ppm, respectively. The "no effect" concentration was 27.7 ppm. Additional information concerning this test was not available.

The 48-hour static LC_{50} of toluene to larvae of the Pacific oyster (Crassostrea gigas) was reported to be 1050 mg/ℓ (LeGore, 1974). This test was conducted with filtered seawater (25.3 to 30.8 ^{o}/oo salinity) at 20 to 21.5°C using 30,000 larvae per exposure concentration.

17.3.2. Sublethal Effects.

17.3.2.1. FISH -- Very little information is available concerning the sublethal effects of toluene exposure on fish. Morrow et al. (1975) studied the effects of several aromatic hydrocarbons, including toluene, on the levels of Na^+ and K^+ in the blood of young coho salmon (Oncorhynchus kisutch) in seawater. Static exposure to 30 ppm toluene caused a small increase in these blood cations, reaching a maximum at about 2 hours after beginning exposure. The Na^+ concentration returned to the control level by 3 hours. Blood K^+ decreased after 2 hours but was still elevated at 4 hours, the last sampling period. The toluene exposure concentration of 30 ppm was sufficient to cause some mortalities and behavioral effects. The authors suggested that toluene increased membrane permeability, particularly in the gills. In the hypertonic seawater medium, this change would result in ion influx and water loss in the fish, perhaps accounting for the initial rise in blood ion concentration.

Brenniman et al. (1979) conducted a series of experiments to determine the effects of toluene exposure on blood gas physiology, hippuric acid content, and histopathology of goldfish (Carassius auratus). The fish used in these experiments were exposed to two or more toluene concentrations under flow-through conditions using dechlorinated tapwater.

For the pathology study, groups of six fish were exposed for up to 30 days to 0, 5, 10, and 21 ppm toluene (Brenniman et al., 1979). No gross or microscopic lesions were observed in fish during the first week of exposure. After the first week, ascites developed in 3 fish at 21 ppm and in 2 fish at 10 ppm. In exposed fish that survived 15 to 30 days, about 50% had a white epidermal exudate of unknown origin, and some fish at all toluene concentrations had gross lesions in gill, liver, or gall bladder. Excessive mucus production in gills occurred in all fish at 21 and 10 ppm and in 50% of the fish at 5 ppm. Microscopic lesions were found in gills (fusion), liver (decreased cytoplasmic nuclear ratio), and kidney (tubular vacuolization) of many exposed fish but not in control fish. Exposed fish did not eat food and had livers that were paler and smaller than control fish.

For the blood gas study, groups of 3 or 4 fish were exposed for 4 hours to 0, 60, or 80 ppm toluene (Brenniman et al., 1979). The blood samples were analyzed for pH, percent oxygen saturation, partial pressures of carbon dioxide (p_{CO_2}) and oxygen (p_{O_2}), and bicarbonate. The results are presented below:

Toluene Conc. (ppm)	Mean Values				
	p_{O_2}	p_{CO_2}	pH	O_2-Saturation (%)	Bicarbonate
0	42.33	11.50	7.56	48.67	9.83
60	16.25[a]	23.25[a]	6.90[a]	27.00[a]	5.10
80	15.63[a]	19.27	6.96[a]	20.33[a]	4.17[a]

[a] $P < 0.05$ when compared to control.

Toluene exposure caused significant changes in all parameters (Brenniman et al., 1979). The authors suggested that the decreased p_{O_2}, increased p_{CO_2}, and resultant acid-base imbalance may have been due to lowered O_2 and CO_2 exchange at the gills. Two proposed mechanisms for impaired gas exchange were lowered respiratory rate and gill damage. The former mechanism is less likely because sublethal toluene exposure has been shown to increase the respiratory rate in fish (Sloof, 1978, 1979; Thomas and Rice, 1979). The latter mechanism is supported by the authors' observation that toluene caused excess mucus production and fusion of gill lamellae in gills.

The whole-fish content of hippuric acid was measured in fish exposed in groups of 6 fish to 0, 5, 10, or 21 ppm toluene for 96 hours (Brenniman et al., 1979). This experiment was conducted to determine whether the fish were able to metabolize toluene ultimately to hippuric acid, as occurs in mammals (Chapter 12.). The results, presented below, indicated that hippuric acid was elevated at all the toluene concentrations tested and that this metabolic pathway occurs in goldfish.

Toluene Concentration (ppm)	Mean Hippuric Acid Concentration (ppm)
0	1539.50
5	3608.67[a]
10	3536.67[a]
21	2829.17[a]

[a] $P < 0.05$ when compared to control.

The pattern of decreasing hippuric acid concentration with increasing toluene concentration was attributed to increasing stress and lower metabolic efficiency as toluene concentration increased. Hippuric acid was elevated above the control levels, however, even at the highest toluene concentration.

The only other information available relevant to toluene metabolism in fish is provided by Ohmori et al. (1975), who investigated the comparative *in vitro* metabolism of a toluene analog, *p*-nitrotoluene, by liver homogenates of rats and eels. The species of eel was not specified. Both species were able to metabolize *p*-nitrotoluene (PNT) to *p*-nitrobenzoic acid (PNB acid), via oxygenation of PNT to *p*-nitrobenzyl alcohol (PNB alcohol), to *p*-nitrobenzaldehyde (PNB aldehyde), and finally to PNB acid. The rate of the overall reaction (PNT to PNB acid) in eel liver, however, was only 34% (at 25°C) to 46% (at 37°C) of the rate in rat liver. The rate of formation of PNB alcohol from PNT in eel liver was 29% (at 25°C) to 16% (at 37°C) of the rate in rat liver. This step was the rate-limiting step for the overall reaction because the formation of PNB acid from PNB alcohol was faster in eels than in rats.

Thomas and Rice (1979) measured the effects of flow-through toluene exposure on the respiratory rate and oxygen consumption of pink salmon (*Oncorhynchus gorbuscha*) fry at two temperatures (4°C, 12°C) in seawater. The fish were placed in sealed chambers fitted with a water inlet and outlet, mesh electrodes (for measuring opercular breathing rate), and oxygen electrodes (for measuring oxygen concentration of inflowing and outflowing water). After determining the 24-hour LC_{50} (5.38 ppm), the authors exposed fry to several toluene concentrations, expressed as percentages of the LC_{50}. Significant increases in opercular breathing rate at 12°C occurred at exposure concentrations of 94 and 69% of the LC_{50}, but not at 45 or 30% of the LC_{50}. The breathing rate remained elevated throughout the 15-hour exposure period only at 94% of the LC_{50}, at which concentration 6 of 23 fish died. The breathing rate at a toluene exposure concentration of 69% of the LC_{50} reached a maximum at 3 hours and returned to control level by 15 hours. Additional experiments showed that exposures to 71% of the LC_{50} increased oxygen consumption. The percent increase in both oxygen consumption and breathing rate was greater at 4°C than at 12°C. The authors suggested that these effects were due to the energy requirements for metabolism of toluene and that this requirement was greater at the lower temperature. The threshold for an effect on breathing rate at 12°C was estimated to be about 46% of the LC_{50}, or about 2.5 ppm.

Sloof (1978, 1979) conducted similar experiments to determine the sensitivity of a biological monitoring system using fish respiratory rates as an indicator of water pollution by toluene and other chemicals. Adult rainbow trout (mean weight 56 g) were acclimated to dechlorinated tapwater at 20 ± 1°C and tested individually in sealed flow-through chambers equipped with stainless steel mesh electrodes for measuring breathing rate. After the normal breathing rate for a fish over a 3 day period had been determined, toluene contaminated water was added continuously and the breathing rates were monitored over a period of 48 hours. Measurements were taken at the same time of day during the pre-exposure and exposure periods. A toxic effect was considered to have occurred if the respiration frequency of at least 75% of the test fish exceeded the predetermined individual normal frequencies measured at the same hourly interval. The lowest toluene concentration that caused an increase in respiratory rate was 2.5 mg/ℓ. This concentration is identical to the estimated threshold concentration for an effect on breathing rate in pink salmon (Thomas and Rice, 1979).

Leung and Bulkley (1979) investigated the effects of 100 μℓ/ℓ toluene on the rate of opercular movement by 8-day-old embryos of the Japanese medaka, Oryzias medaka. The basal (unexposed) rate was determined for each of three embryos and then toluene was added to the culture medium to obtain a nominal concentration of 100 μℓ/ℓ. The rate was then determined for each embryo at about 5 minute intervals for 40 minutes. The average rate before exposure was zero movements/minute. The average of 8 counts (each 1 minute long) over 40 minutes after beginning exposure was 2.28 movements/minute. The standard deviation was so great, however, that this increase was not statistically significant.

The sublethal effects of toluene on medaka were also investigated by Stoss and Haines (1979). The exposure techniques and lethal effects reported by these authors have been discussed in Section 17.3.1.1. Static exposure of eggs to initial nominal concentrations of 41 and 82 mg toluene/ℓ resulted in a significant delay in time to hatching and a decrease in the proportion of embryos that hatched successfully. Exposure to 41 mg/ℓ and greater caused numerous developmental abnormalities, including disruption of cell cleavage patterns, deformation of eyes, appearance of isolated blood islands in the circulatory system, and abnormal heart structure, tail flexures, and visceral organ formation and placement. No abnormalities were observed in embryos exposed to 16 mg toluene/ℓ.

Subchronic embryolarval toxicity tests have been conducted with freshwater fathead minnows (Devlin et al., 1981) and saltwater sheephead minnows (Ward et al., 1981). Devlin et al. (1982) exposed embryos under flow-through conditions to 4 to 15 mg/ℓ toluene for 36 days. Larval growth was the most sensitive indicator of toxicity, with significant inhibition of larval growth occurring at toluene concentrations as low as 6 mg/ℓ. No effects were observed at 4 mg/ℓ. The maximum acceptable toxicant concentration (MATC) was, therefore, between 4 and 6 mg/ℓ for this species.

Ward et al. (1981) conducted flow-through toxicity tests with embryos of sheepshead minnows exposed to 1 to 19 mg/ℓ toluene for 28 days after hatching. Exposure to 7.7 mg/ℓ and greater caused a significant decrease in hatching success and survival of juveniles. There were no effects on growth of surviving fish. As a result, the MATC was >3.2 <7.7 mg/ℓ. The 96-hour LC_{50} for this species was between 277 and 485 ppm (Section 17.3.1.2.). The ratio between acute and sub-chronic toxicity was between 36 and 152, indicating that chronic effects occur at concentrations much lower than acute effects.

In summary, the lowest toluene concentration shown to cause sublethal effects in fish was 2.5 ppm, the concentration that caused an increased breathing rate in trout (Sloof, 1978, 1979) and salmon (Thomas and Rice, 1979). This value is somewhat below the lowest acute LC_{50} value reported for any fish species (3.08 ppm for coho salmon, see Table 17-1). An embryo-larval test with sheepshead minnows (U.S. EPA, 1980) showed that subchronic toxic effects occurred at 7.7 ppm but not at 3.2 ppm and that the ratio of the acute LC_{50} to the subchronic MATC for this species was between 1.7 and 4.0. Another embryo-larval test with fathead minnows (Devlin et al., 1982) showed that subchronic effects occurred at 6 mg/ℓ but not at 4 mg/ℓ, and that the ratio of the acute LC_{50} to the subchronic MATC was between 3 and 18. Although acute-chronic ratios may vary greatly among species, this information suggests that chronic toxic effects may occur in coho salmon and other sensitive species at concentrations well below 3 ppm.

17.3.2.2. INVERTEBRATES -- Berry et al. (1978) conducted a series of experiments to determine the effects of 24 hours of exposure to sublethal concentrations of water-soluble fractions (WSFs) of gasoline, benzene, xylenes, and toluene on oxygen consumption by fed and unfed larval stages of the mosquito, Aedes aegypti. Control experiments with untreated animals showed that there was no significant difference in O_2 consumption between fed and unfed larvae. Treatment with the WSF of 1 mℓ/ℓ gasoline, however caused an increased O_2 consumption in fed, but not unfed, larvae relative to untreated controls. Treatment of fed larvae with individual WSFs of benzene (1 mℓ/ℓ), xylenes (0.3 mℓ/ℓ), or toluene (0.1 to 0.5 mℓ/ℓ) had no effect on O_2 consumption relative to fed controls. A WSF mixture of benzene, xylenes, and toluene and a mixture of benzene and toluene

(0.2 mℓ/ℓ for each compound) caused significant increases in O_2 consumption. Exposure to a WSF mixture of benzene and xylenes or toluene and xylenes (0.2 mℓ/ℓ for each compound) had no effect. The authors also conducted experiments on the uptake of ^3H-labeled toluene in fed and unfed animals, as well as uptake of ^3H-toluene by fed larvae in the presence or absence of benzene (Section 15.3.). Maximum ^3H-toluene counts were equal in fed and unfed larvae, but were reached more quickly (1 hour versus 4 hours) by the fed animals. The ^3H-toluene counts in larvae, expressed as the percentage of the initial water counts, were greater in the benzene and toluene mixture than in the solution containing toluene alone. The authors concluded that the effects of gasoline on O_2 consumption were due to the enhanced uptake and synergistic effects of toluene and benzene, two of the major aromatic components of gasoline. They also suggested that the presence of food accelerated the uptake of toluene through absorption of toluene to the consumed food particles.

Blundo (1978) investigated the effects of toluene on the swimming activity and survival of barnacle (Balanus eburneus) larvae. Groups of larvae were exposed for 1 hour in specially constructed tubes to 10, 20, 30, 40, 50, 60, 70, 80, and 90% of the water soluble fraction (WSF) made by saturating seawater with toluene. The tubes were designed so that actively swimming photopositive larvae would be attracted to light at the top of the tube. After 1 hour of exposure, the inactive larvae were collected from the bottom of the tubes and stained with a vital dye (neutral red) to determine percent mortality. The remaining portion, containing the active larvae, was then collected and counted. The interpolated concentration that immobilized 50% of the larvae was 12.5% of the WSF. All larvae were immobilized at 30% WSF and higher. About 33-1/3% of the larvae were immobilized at 10% WSF, the lowest concentration tested. The percent mortality of the immobilized larvae ranged from about 3% at 10% WSF to a maximum of 12% at 90% WSF. The author also measured the effects of WSFs that had been aged in covered containers for 1 day in a refrigerator or exposed to air for up to 3 days. The percent WSF that immobilized 33-1/3% of the larvae was 10% in the fresh solution, 37.5% in the refrigerated solution, and 90% in the evaporated solution. Additional experiments showed that aeration of the WSF for 6 hours lowered the toxicity to the same extent as 3 days of exposure to air.

Bakke and Skjoldal (1979) investigated the effects of toluene on activity, survival, and physiology of the isopod, Cirolana borealis. For determination of median effective times (ET_{50}, partial or complete narcotization as endpoint), groups of 15 isopods were exposed in duplicate to nominal initial concentrations of 0, 0.0125, 1.25, 5.7, 12.5, 25, and 125 ppm toluene for 4 days. The exposure medium (33.5 to 34.5 °/oo salinity seawater at 8 to 10°C) was changed every 2 days. The interpolated or extrapolated ET50 values were as follows:

Toluene Concentration (ppm)	ET_{50} (hours)
0	---
0.0125	---
1.25	---
5.7	400
12.5	69
25	28
125	3

No effects on activity were observed in animals exposed to 1.25 ppm or less (Bakke and Skjoldal, 1979). The authors also investigated the recovery of isopods after exposure for varying periods to 12.5 or 125 ppm toluene. Exposure to 125 ppm for 1 hour caused complete inactivity, but all animals recovered within 12 hours after transfer to clean water. Exposure for 2 or more hours to 125 ppm caused partial or complete mortality. All isopods could recover after exposure to 12.5 ppm for 30 hours but not longer. Additional experiments showed that there was no significant effect of 4 days of exposure to up to 5.7 ppm toluene on oxygen consumption, ATP concentration, or energy charge. Exposure to 12.5 ppm resulted in a progressive decrease in ATP level and energy charge over 8 days of exposure, at which time all organisms had died. Exposure to the rapidly lethal concentration of 125 ppm toluene showed no effect on ATP level or energy charge. These results with 12.5 and 125 ppm were essentially the same as those reported by the authors in a previous paper (Skjoldal and Bakke, 1978). Bakke and Skjoldal (1979) concluded that the effect of toluene on activity was much more sensitive as an indicator of sublethal toluene toxicity than its effects on respiration, ATP level, and energy charge.

In summary, the lowest toluene concentration shown to cause sublethal effects in invertebrates was 5.7 ppm, the concentration that caused narcotization of isopods (Bakke and Skjoldal, 1979). This concentration is somewhat higher than the 96-hour LC_{50} of 4.3 ppm for bay shrimp (see Table 17-1) reported by Benville and Korn (1977). The latter concentration is the lowest reported to have toxic effects on freshwater or marine invertebrates. Although the chronic toxicity of toluene to aquatic invertebrates has not been studied, it is probable that chronic effects could occur in sensitive invertebrate species at concentration below 4.3 ppm. This conclusion is supported by the fact that chronic effects in fish occurred at concentrations well below the acutely toxic concentrations (Section 17.3.2.2.).

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18. HEALTH EFFECTS SUMMARY

18.1. EXISTING GUIDELINES AND STANDARDS

18.1.1. **Air.** The Occupational Safety and Health Administration (OSHA) currently limits occupational exposure to toluene to 200 ppm as an 8-hour, time-weighted-average (TWA), with an acceptable ceiling concentration of 300 ppm (40 CFR 1910.1000); the acceptable maximum peak above the ceiling concentration is 500 ppm for a maximum duration of 10 minutes. The National Institute for Occupational Safety and Health (NIOSH, 1973) currently recommends an exposure limit of 100 ppm as an 8-hour TWA with a ceiling of 200 ppm. An 8-hour TWA concentration of 100 ppm is also recommended by the American Conference of Governmental Industrial Hygienists (ACGIH, 1980) as a Threshold Limit Value (TLV) for toluene; the short-term (15 minute) exposure limit recommended by the ACGIH is 150 ppm. ACGIH (1980) has further noted that there may be significant contribution to the overall exposure by the cutaneous route.

Threshold limit values that have been established for occupational exposure to toluene in other countries are listed as follows (Verschueren, 1977):

USSR	13 ppm (50 mg/m^3)	1972
Czechoslavakia	52 ppm (200 mg/m^3)	1969
West Germany (BDR)	200 ppm (750 mg/m^3)	1974
East Germany (DDR)	52 ppm (200 mg/m^3)	1973
Sweden	98 ppm (375 mg/m^3)	1975

There are no standards for general atmospheric pollution by toluene in the United States, although a National Ambient Air Quality Standard specifies that nonmethane hydrocarbons shall not exceed 0.24 ppm (160 $\mu g/m^3$) as a maximum 3-hour average concentration (6 to 9 a.m.), more than once per year (40 CFR 50). Ambient air quality standards have, however, been promulgated for toluene in other countries. These foreign standards are summarized as follows (Verschueren, 1977):

Country	Concentration	Averaging Time
USSR	0.15 ppm (0.6 mg/m^3)	20 min
	0.15 ppm (0.6 mg/m^3)	24 hr
West Germany (BRD)	15 ppm (60 mg/m^3)	30 min
	5 ppm (20 mg/m^3)	24 hr

East Germany (DDR)	0.5 ppm (2.0 mg/m^3)	30 min
	0.15 ppm (0.6 mg/m^3)	24 hr
Bulgaria	0.15 ppm (0.6 mg/m^3)	20 min
	0.15 ppm (0.6 mg/m^3)	24 hr
Hungary	13.3 ppm (50.0 mg/m^3)	30 min
	5.3 ppm (20.0 mg/m^3)	24 hr
Hungary (protected areas)	0.16 ppm (0.6 mg/m^3)	30 min
	0.16 ppm (0.6 mg/m^3)	24 hr
Yugoslavia	0.16 ppm (0.6 mg/m^3)	20 min
	0.16 ppm (0.6 mg/m^3)	24 hr

18.1.2. Water. The Committee on Safe Drinking Water of the National Academy of Sciences concluded in 1977 that toluene and its major metabolite, benzoic acid, were relatively nontoxic, and that there was insufficient toxicological data available to serve as a basis for setting a long-term ingestion standard (NAS, 1977). It was recommended that studies be conducted to produce relevant information. Toluene has recently been considered for a second time by a reorganized Toxicology Subcommittee of the Safety Drinking Water Committee of the National Academy of Sciences (U.S. EPA, 1980a), but the results of the deliberations of this group have not yet been made public.

The U.S. EPA (1980a) has recently derived an ambient water criterion level for toluene of 14.3 mg/ℓ. This criterion is intended to protect humans against the toxic effects of toluene ingested through water and contaminated aquatic organisms, and is based on an Acceptable Daily Intake (ADI) calculated from the maximum-no-effect dose reported in the Wolf et al. (1956) subchronic oral study in rats and an uncertainty factor of 1000. The criterion level for toluene can alternatively be expressed as 424 mg/ℓ if exposure is assumed to be from the consumption of fish and shellfish products alone.

18.1.3. Food. Toluene has been approved by the Food and Drug Administration for use as a component of articles intended for use in contact with food (i.e., an indirect food additive). Articles that contain residues of toluene may be used in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food. The use of toluene in the food industry is summarized as follows:

Component of adhesives	21 CFR 175.105
Adjuvant substance in resinous and polymeric coatings for polyolefin films used as food contact surfaces	21 CFR 175.320

Component of the uncoated or coated surfaces of paper and paperboard articles intended for use with dry foods	21 CFR 176.180
Used in the formulation of semirigid and rigid acrylic and modified acrylic plastic articles	21 CFR 177.1010
Additive for cellophane (residue limit 0.1%)	21 CFR 177.1200
Additive for 1,4-cyclohexylene dimethylene terephthalate and 1,4-cyclohexylene dimethylene isophthalate copolymer	21 CFR 172.1240
Solvent for 4,4'-isopropylidenediphenol-epichlorohydrin resins with a minimum molecular weight of 10,000 (residue limit ≤1000 ppm in the finished resin)	21 CFR 177.1440
Solvent for polysulfide polymer-polyepoxy resins	21 CFR 177.1650
Solvent for poly(2,6-dimethyl-1,4-phenylene)oxide resins (residue limit 0.2% by weight)	21 CFR 177.2460
Blowing agent adjuvant used in the manufacture of foamed polystyrene (residue limit ≤0.35% by weight of finished foamed polystyrene)	21 CFR 178.3010

Toluene has also been exempted from the requirement of a tolerance when it is used as a solvent or cosolvent in pesticide formulations that are applied to growing crops (40 CFR 180.1001).

18.2. INHALATION EXPOSURES

As detailed in Chapter 11 of this report, many studies have reported the effects on humans of inhalation exposures to toluene. Because most of these studies involved relatively small numbers of human subjects, they failed to precisely define the levels or durations of the exposures, and/or did not consider the potential role of exposures to other toxicants. None of these studies would be suitable for human risk assessment if taken individually. In combination, however, they constitute a considerable body of human experience and provide a relatively consistent pattern of dose-response relationships.

18.2.1. Effects of Single Exposures. The effects on humans of single exposures to toluene for periods of up to 8 hours are relatively well documented. Data on both toluene glue sniffers (Press and Done, 1967a, 1967b; Wyse, 1973; Lewis and Patterson, 1974; Helliwell and Murphy, 1979; Hayden et al., 1977; Oliver and Watson, 1977; Barnes, 1979) and workers accidentally exposed to high levels of toluene (Lurie, 1949; Browning, 1965; Longley et al., 1967; Reisen et al., 1975) indicate that exposure to air saturated or nearly saturated with toluene can cause a spectrum of effects, from lightheadedness to unconsciousness, in a very short period of time. Deaths attributed to the deliberate inhalation of toluene have been reported in at least 24 cases (Winek et al., 1968; Chiba, 1969; Nomiyama and Nomiyama, 1978). Although most of these reports do not provide quantitative exposure estimates, glue sniffers are probably exposed to nearly saturated air-vapor mixtures of about 30,000 ppm toluene. The occupational report of Longley et al. (1967) indicated that a loss of consciousness occurred within minutes after exposure to atmospheres estimated to contain 10,000 ppm toluene at waist level and 30,000 ppm toluene at floor level. The acute inhalation toxicity data on experimental mammals, summarized in Table 12-1, suggest that exposure periods of several hours to toluene levels greater than 4000 ppm may be lethal. Based on the results of longer term human studies discussed below, short exposures to concentrations of up to 1500 ppm are not likely to be lethal (Wilson, 1943; Ogata et al., 1970, see following discussion). The single report by Gusev (1965) of effects on EEG activity in 4 individuals exposed to 0.27 ppm for 6 minute intervals may be a subtle indication of the perception of toluene at this low level but does not have any apparent toxicologic significance.

For single exposure periods that approximate a normal working day (7 to 8 hours), von Oettingen et al. (1942a, 1942b) and Carpenter et al. (1944) provide relatively consistent information on sublethal dose-response relationships. As summarized previously in Table 10-1, von Oettingen et al. (1942a, 1942b) noted a range of subjective complaints from 8 hour exposures to toluene concentrations ranging from 50 ppm (drowsiness) to 800 ppm (severe fatigue, nausea, incoordination, etc., with after effects lasting at least several days). Although the terminology used by Carpenter et al. (1944) is somewhat different from that used by von Oettingen, the effects noted seem comparable over the common exposure range (200 to 800 ppm). Although the consistency between these two studies is reassuring, it should be noted that, even combined, both studies involve exposures of only five individuals who were placed on multiple exposure/recovery schedules. The impact that such multiple exposures could potentially have on the results cannot be determined. Given the small number of individuals involved in the exposures to toluene, an attempt to generalize for the human population a detailed dose-response gradient comparable to that presented in Table 11-1 does not seem justifiable. When these studies are considered along with the results of Ogata and coworkers (1970) and Gamberale and Hultengren (1972), however, it seems reasonable to conclude that exposure periods of 8 hours or less to toluene concentrations below 100 ppm may result in mild subjective complaints (fatigue or headache) but are not likely to induce observable effects. Concentrations above 100 ppm may cause impaired reaction time (200 ppm x 3 hours, Ogata et al., 1970; 300 ppm x 20 minutes, Gamberale and Hultengren, 1972). At concentrations of 300 to 800 ppm and above, gross signs of incoordination may be expected (von Oettingen et al., 1942a, 1942b; Carpenter et al., 1944).

Accidental acute overexposure to toluene may be limited to some extent by the organoleptic or irritant properties of the compound. Gusev (1965) reports ranges of maximum imperceptible concentrations and minimum perceptible concentrations of 0.35 to 0.79 ppm and 0.40 to 0.85 ppm, respectively. May (1966) reports a minimum perceptible concentration of 37 ppm. The reasons for this discrepancy between the Russian and American values are not apparent. Although the Russian study entailed a total of 30 subjects and 744 observations and the American report involved 16 individuals (number of observations not specified), it is unlikely that the difference in the reported detectable levels is due simply to sample size. In any event, toluene appears to be detectable in the air at levels below those causing impaired coordination (i.e., >100 ppm). In addition, Carpenter and coworkers (1944) reported that toluene caused mild throat and eye irritation at 200 ppm and also caused lacrimation at 400 ppm.

In summary, the estimated dose-response relationships for the acute effects of single short-term exposures to toluene are presented below:

10,000 to 30,000 ppm : Onset of narcosis within a few minutes. Longer exposures may be lethal.

>4,000 ppm : Would probably cause rapid impairment of reaction time and coordination. Exposures of 1 hour or longer might lead to narcosis and possibly death.

1,500 ppm : Probably not lethal for exposure periods of up to 8 hours.

300 to 800 ppm : Gross signs of incoordination may be expected during exposure periods up to 8 hours.

400 ppm : Lacrimation and irritation to the eyes and throat.

100 to 300 ppm : Detectable signs of incoordination may be expected during exposure periods up to 8 hours.

200 ppm : Mild throat and eye irritation.

50 to 100 ppm : Subjective complaints (fatigue or headache) but probably no observable impairment of reaction time or coordination.

>37 ppm : Probably perceptible to most humans.

From the above discussion, it should be evident that these approximations are crude composites and contain several areas of uncertainty and overlap.

18.2.2. Effects of Intermittent Exposures Over Prolonged Periods. Limited information is available on the effects of subchronic or chronic continuous exposures to toluene on humans or experimental animals. Most of the studies either involve occupational exposures or are designed to mimic occupational exposures. Consequently, while the data described below may be directly applicable to estimating effects from occupational exposures, an additional element of uncertainty must be considered in any attempt to estimate the effects of continuous exposures that may occur from ambient air.

Wilson (1943) provides the only acceptable data on the effects of repeated occupational exposures to toluene over a period of weeks (Section 11.1.1.2.). In this study, the workers were classified into three groups by the levels of toluene to which they were exposed: 50 to 200 ppm, 200 to 500 ppm, and 500 to 1500 ppm. The effects noted at the various levels were essentially the same as those seen in single exposures. In the low exposure group, the reports of headache and lassitude are consistent with symptoms noted by von Oettingen and coworkers (1942a, 1942b) over the same range of exposure. Although Wilson (1943) did not attribute these effects to toluene exposure, his failure to include an unexposed control group makes this judgment questionable in view of the von Oettingen data. In the middle and high exposure groups, the reports of headache, nausea, and concentration-related impairment of coordination and reaction time are also consistent with the symptoms reported by von Oettingen and coworkers (1942a, 1942b) and Carpenter and coworkers (1944) for short-term single exposures. The major discomforting feature of the Wilson (1943) report is that it involved only 100 out of a total of 1000 workers. It is unclear whether the remaining 900 workers evidenced any symptoms of toluene exposure.

The only other study that reports effects of repeated exposures to toluene for relatively short periods of time is that presented by Greenburg and coworkers (1942). In this study, repeated occupational exposures to toluene at levels of 100 to 1100 ppm for periods of 2 weeks to 5 years were associated with enlarged livers in 13 of 61 airplane painters. This incidence of liver enlargement was reported to be 3 times that of a control group of 430 workers not exposed to toluene. Because Greenburg and coworkers (1942) were not able to associate liver enlargement with clinical or laboratory evidence of disease, because the painters were also exposed to significant quantities of other volatile paint components (Table 11-9), and because the liver effect has not been corroborated by other investigators (e.g., Parmeggiani and Sassi, 1954; Suhr, 1975), the hepatomegaly reported by Greenburg should be given relatively little weight in risk assessment.

Other reports of repeated occupational exposures to toluene involve periods of several years. For mean exposure levels above 200 ppm, all of the available studies except that of Suhr (1975) report some evidence of neurologic effects (Capellini and Alessio, 1971; Parmeggiani and Sassi, 1954; Munchinger, 1963; Rouskova, 1975).

The Suhr (1975) study involved a group of 100 printers exposed to 200 to 400 ppm toluene for over 10 years. Compared to a group of 100 non-exposed individuals, no significant differences were seen in symptoms of CNS depression or sphallograph tests, which are designed to measure muscular coordination. An interpretation of the significance of the Suhr (1975) study is confounded, however, by several factors. As discussed in Sections 11.1.1.2. and 11.3., the limitations of this study include an undefined control group, uncertainties involving the time of reflex reaction and sphallograph testing (i.e., blood toluene levels may have declined significantly if the workers were examined before or after the work shifts), and the use of an apparently unvalidated device (sphallograph) for the detection of slight disturbances of muscular coordination.

The other studies that do report effects at equal or higher levels of exposure can be challenged for various reasons. The report of "nervous hyper-excitability" in 6 of 11 exposed to 200 to 800 ppm toluene for "many years" (Parmeggiani and Sassi, 1954) does not seem to be characteristic of toluene intoxication. This report is from the Italian literature, however, and a full text translation has not yet been made available for this review. The Capellini and Alessio (1971) study, which associated stupor, nervousness, and insomnia with occupational exposure to 250 (210 to 300) ppm toluene for several years, involved only a single worker. The "organic psychosyndrome" diagnosed by Munchinger (1963) in workers exposed to 300 and 430 ppm toluene for 18 and 12 years, respectively, is supported by the results of Rorschach tests and Knoepfel's 13-Error tests. Because Munchinger did not use a control group, however, the utility of this study is limited. The changes in EEG response to photic stimulation that were reported by Rouskova (1975) in workers exposed to >250 ppm toluene for an average of 13.5 years also involved exposure to unspecified levels of 1,1,1-trichloroethane. Thus, the interpretation of the discrepancies between the study by Suhr (1975) and these other reports is problematic. Considering the relatively well documented CNS effects of single exposures to toluene at levels above 200 ppm (Section 18.1.1.) and the effects noted by Wilson (1943) at comparable levels for much shorter periods of time, it would seem imprudent to accept the Suhr (1975) data as a "no-observed-effect level" for human risk assessment.

An alternative approach could be to use the study by Capellini and Alessio (1971) in which no CNS or liver effects were noted in a group of 17 workers occupationally exposed to 125 (80 to 160) ppm toluene for "diverse years." In addition to the problems of small sample size, failure to precisely define the duration of exposure, and lack of a control group, the use of this study is compromised by reports of effects in two other groups of workers at lower levels of toluene exposure. Matsushita and coworkers (1975) reported impaired performance in neurological and muscular function tests in a group of 38 female shoemakers who had been exposed to 15 to 200 ppm toluene for an average of 3 years and 4 months. In addition, 19 of 38 exposed women, compared to 3 of 16 in the control group, complained of dysmenorrhea. The second group of workers was composed of 100 car painters who had been occupationally exposed to an average of 30.6 ppm toluene for an average of 14.8 years. As reported by Hanninen and coworkers (1976) and Seppalainen and coworkers (1978), the exposed workers had a greater incidence of CNS symptoms and impaired performance on tests for intelligence and memory, as well as for visual and verbal ability. Both of the studies on this group of workers used control groups of approximately 100 unexposed individuals. The major problem with the reports of adverse effects on the female shoemakers and male car painters is that both groups were exposed to other potentially toxic agents. The female shoemakers were exposed to "slight" levels of gasoline (Matsushita et al., 1975) and, as detailed in Table 11-3, the male car painters were exposed to several other organic solvents.

The subchronic and chronic data on experimental mammals are of only limited use in helping to resolve the uncertainties in the human data. Jenkins and coworkers (1970), and CIIT (1980) report no-observable-effect levels (NOELs) in experimental mammals 1085 ppm (8 hours per day, 5 days per week for 6 weeks) and 300 ppm (6 hours per day, 5 days per week for 24 months), respectively. As discussed above in this section, a NOEL of 1085 ppm is contradicted by human experience, suggesting that humans are more sensitive than experimental mammals to toluene exposure. Similarly, the continuous-exposure NOEL of 107 ppm for 90 days in rats, guinea pigs, dogs, and monkeys (Jenkins et al., 1970), and the 2-year intermittant exposure NOELS of 30 ppm and 100 ppm in rats (CIIT, 1980), do not, by themselves, negate the concerns with neurological effects reported in humans at lower levels.

18.3. ORAL EXPOSURES

Very little information is available on the acute, subchronic, or chronic effects of toluene in experimental mammals. As summarized in Table 12-1, acute oral LD_{50}s in adult rats range from 5500 mg/kg to 7530 mg/kg. Using the cubed root of the body weight ratios for interspecies conversion (U.S. EPA, 1980b; Freireich et al., 1966; Rall, 1969), an approximate lethal dose for humans can be estimated at 983 mg/kg (5500 mg/kg $\cdot$ (70 kg $\cdot$ 0.4 kg)$^{1/3}$). The conversion factor, as used here, assumes that humans are more sensitive than rats, which, as discussed above, is consistent with the available data on inhalation exposure. This estimate of the approximate lethal dose is also consistent with the report by Francone and Braier (1954) that leukemia patients were able to tolerate cumulative doses of up to 130,000 mg of toluene given over a 3-week period (approximately 88 mg/kg/day).

The only subchronic oral data are reported in the study by Wolf and coworkers (1956), indicating a NOEL in rats at 590 mg/kg/day, given 5 days per week for 6 months.

18.4. DERMAL EXPOSURES

Studies on the dermal toxicity of toluene are inadequate for quantitative risk assessment. Qualitatively, the little information that is available suggests that moderate dermal contact with liquid toluene (i.e., exposure of human forearm skin to toluene for 1 hour on 6 successive days) may cause skin damage but does not result in overt signs of toxicity (Malten et al., 1968). Similarly, the acute and subchronic data on toluene exposure in experimental mammals do not suggest that toluene is a potent toxicant on dermal contact. A method for quantitatively using such data to estimate equivalent human dose-response relationships, however, has not been fully formulated or validated.

As discussed in Section 13.1., exposure to toluene vapor results in relatively little dermal absorption compared to absorption across the lungs.

18.5. RESPONSES OF SPECIAL CONCERN

18.5.1. Carcinogenicity. CIIT (1980) concluded that exposure to 30, 100, or 300 ppm toluene for 24 months did not produce an increased incidence of neoplastic, proliferative, inflammatory, or degenerative lesions in Fischer 344 rats. It should be noted, however, that this study has been considered inadequate for carcinogenicity evaluation because the highest level tested was not a maximum tolerated dose. Also, the high spontaneous incidence (16%) of mononuclear cell leukemia in aging Fischer 344 male rats reported by Coleman and coworkers (1977) suggests that this strain may be inappropriate for the study of a chemical that might be myelotoxic.

Other studies suggest that toluene is not carcinogenic when applied topically to the shaved skin of animals. Toluene is used extensively as a solvent for lipophilic chemicals being tested for carcinogenic potential; negative control studies employing 100% toluene have not elicited carcinogenic effects. Also, no evidence of a promotion effect was noted when toluene was painted on the skin of mice twice weekly for 20 weeks following initiation with 7,12-dimethylbenz-[a]-anthracene (Frei and Stephens, 1968; Frei and Kingsley, 1968).

The above data are not adequate for assessing the potential carcinogenicity of toluene with great assurance and they cannot be used for supporting carcinogenicity as a valid biologic endpoint in quantitative risk assessment.

18.5.2. Mutagenicity. Toluene has yielded negative results in a battery of microbial, mammalian cell, and whole organism test systems as indicated in the following:

Differential Toxicity/DNA Repair Assays
  Escherichia coli
  Salmonella typhimurium

Reverse Mutation Testing
  Salmonella typhimurium (Ames test)
  Escherichia coli WP2 assay
  Saccharomyces cerevisiae D7

Mitotic Gene Conversion/Crossing Over
    Saccharomyces cerevisiae D4, D7

Thymidine Kinase Assay
    L5178Y mouse lymphoma cells

Micronucleus Test
    mouse

Dominant Lethal Assay
    mouse

Assays for sister-chromatid exchange (SCE) and cytogenetic effects in human and animal systems have provided inconsistent results. *In vitro* studies have shown that toluene treatment did not alter SCE frequencies in cultured Chinese hamster ovary cells (Evans and Mitchell, 1980), and that SCEs and chromosome aberrations were not induced in cultured human lymphocytes (Gerner-Smidt and Friedrich, 1978). Increased frequencies of SCEs and/or aberrations in lymphocytes from workers who were chronically exposed to similar levels of toluene have, however, been reported by some investigators (100 to 200 ppm, Funes-Craviota et al., 1977; 200 to 300 ppm, Bauchinger et al., 1982), but not by others (200 to 400 ppm, Forni et al., 1971; 7 to 112 ppm, Maki-Paakkanen et al., 1980). In the Russian literature, chromosome aberrations were reported in the bone marrow cells of rats that were exposed subcutaneously (Dobrokhotov, 1972; Lyapkalo, 1973) and via inhalation (Dobrokhotov and Enikeev, 1977) to toluene, but these findings were not corroborated in a Litton Bionetics, Inc. (1978) study with rats following intraperitoneal injection. Differences in doses employed and experimental design (e.g., numbers of cells scored) may account (at least in part) for the conflicting results, but it should be noted that it is probable that part of the exposure in the Funes-Craviota et al. (1977) study was to benzene-contaminated toluene, and that the purity of the toluene used in the Russian studies was not stated.

18.5.3. Teratogenicity. Toluene was reported in a recent abstract from NIEHS to induce cleft palates at a level of 1.0 mℓ/kg (approximately 866 mg/kg) following oral exposure to mice on days 6 to 15 of gestation (Nawrot and Staples, 1979). This effect reportedly did not appear to be due merely to a general retardation in growth rate. Levels of 0.3 and 0.5 mℓ/kg (approximately 260 and 433 mg/kg) toluene had no teratogenic effect, but the number of mice exposed and number of fetuses examined were not stated. Nawrot and Staples (1979) also noted a significant increase in embryonic lethality at all dose levels and a significant reduction in fetal weight at the two higher dose levels. No frank signs of maternal toxicity were seen at any dose level; however, at the highest dose, decreased maternal weight gain was reported in mice exposed on days 12 to 15 of gestation. A complete copy of this report has not been made available for review but has been submitted for publication.

Three other studies have concluded that toluene is not teratogenic in mice (Hudak and Ungvary, 1978) or rats (Hudak and Ungvary, 1978; Litton Bionetics, 1978; Tatrai et al., 1979) following inhalation exposure. Hudak and Ungvary (1978) and Tatrai et al. (1979) have noted, however, an increased incidence of skeletal anomalies and signs of retarded skeletal development in the rats that were not considered malformations as such. Embryotoxicity was also indicated by low fetal weights in mice and some rats (Hudak and Ungvary, 1978). At the high exposure levels in the study by Hudak and Ungvary (1978), increased maternal mortality was noted in rats (399 ppm, 24 hours/day, days 1 to 8) and mice (399 ppm, 24 hours/day, days 6 to 13). No increased maternal mortality was noted by either Hudak and Ungvary (1978) or Tatrai et al. (1979) at lower exposure levels in rats (266 ppm, 8 hours/day, days 1 to 21; 266 ppm, 24 hours/day, days 7 to 14) or mice (133 ppm, 24 hours/day, days 6 to 13). In the study by Litton Bionetics, Inc. (1978), no signs of maternal toxicity were noted in rats exposed to 100 or 400 ppm, 6 hours/day, on days 6 to 15 of gestation.

The extrapolation of these results to define potential human risk is an uncertain process. The dose that produced cleft palates in mice on oral exposure, 866 mg/kg, is only slightly higher than the NOEL in rats, 590 mg/kg/day.

Although inhalation exposure to toluene has not been shown to be teratogenic, embryotoxicity is an endpoint of concern. The effects noted in rats and mice at the high exposure level (400 ppm) in the study by Hudak and Ungvary (1978) may be of limited use in human risk assessment because of the occurrence of maternal mortality. The lowest effect level not associated with maternal mortality was 133 ppm, 24 hours/day, on days 6 to 13, which caused low fetal weights in mice. No fetal effects were noted in the study by Litton Bionetics, Inc. (1978), however, when rats were exposed to 100 ppm or 400 ppm, 6 hours/day, on days 6 to 15 of gestation, or in the Tatrai et al. (1979) study when rats were continuously exposed to 266 ppm toluene on days 7 to 14. As is the case with oral exposure studies, a quantitative approach for using this type of data in human risk assessment has not been validated.

18.6. REFERENCES

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